BENTON HARBOR POWER PLANT LIMNOLOGICAL STUDIES

PART VI. PONTOPOREIA AFFINIS (CRUSTACEA, AMPHIPODA) AS A MONITOR OF RADIONUCLIDES RELEASED TO LAKE MICHIGAN

Charles C. Kidd

Under Contract with:

Indiana and Michigan Electric Company

Special Report No. 44
of the
Great Lakes Research Division
The University of Michigan
Ann Arbor, Michigan

PONTOPOREIA AFFINIS (CRUSTACEA, AMPHIPODA) AS A MONITOR OF RADIONUCLIDES RELEASED TO LAKE MICHIGAN

Charles C. Kidd

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy in

(Environmental Health)

The University of Michigan

1970

Chairman: G. Hoyt Whipple

ABSTRACT

Present plans call for the building of seven nuclear power plants on the shores of Lake Michigan that will be in operation by 1975 generating approximately 13,000 megawatts (thermal). The liquid wastes discharged to Lake Michigan from these facilities will contain minute quantities of radioactive elements. Accumulation of these radionuclides by aquatic organisms may damage the organisms or pose a radiological health hazard to man. Surveys of the aquatic environment near a nuclear facility must involve sampling of such monitor organisms in order to indicate the extent of damage to the environment and the severity of any health hazard to man. There is a need, therefore, to identify organisms that can be used as monitors of radionuclides released to Lake Michigan. This investigation involves the evaluation of <u>Pontoporeia affinis</u>, a

benthic amphipod native to Lake Michigan, as a monitor of radionuclides found in radioactive wastes.

A review of published information on the ecology of _P. affinis_ in Lake Michigan, and observations of recent sampling results revealed that the average standing crop of this benthic amphipod was approximately 13 g (wet weight) per m^2, and that _P. affinis_ was well distributed in almost all areas of the Lake Michigan benthic community. _P. affinis_ exposed to dilute solutions of radioactive wastes from nuclear facilities accumulated radioactive strontium, manganese and zinc. Chemical analysis of whole _P. affinis_ and lake water by atomic absorption spectrophotometry revealed that naturally occurring concentration factors (based on wet weight) for strontium, manganese and zinc in _P. affinis_ were 260, 5,840 and 3,540 respectively. Repeated exposure of _P. affinis_ to low concentrations (10^{-4} μCi/ml) of manganese -54 and zinc -85 indicated that quasi-equilibrium concentrations of these radionuclides were established in 10 days. Strontium -85 concentrations in _P. affinis_ exposed 10^{-4} μCi/ml approached equilibrium values in 23 days. Variations within the limits normally encountered in the natural environment of temperature and pH did not significantly influence accumulation of radionuclides by _P. affinis_. The presence of natural sediments in the laboratory environments increased the rates of accumulation of manganese-54 and zinc-65 by _P. affinis_. Elimination of accumulated strontium-85, manganese-54 and zinc-65 by _P. affinis_ was prolonged by the presence of radioactive sediment. It was observed that sterilization of natural sediments significantly reduced the accumulation of manganese-54 and zinc-65 by _P. affinis_. It was concluded that _P. affinis_ was a suitable monitor organism for manganese-54 and zinc-65.

Atomic absorption spectrophotometry was used to measure the concentrations of strontium, manganese, and zinc in the flesh of Leuciscus cephalus (chubs), fish which feed almost exclusively on P. affinis. The equilibrium concentration factor for zinc in L. cephalus was 546. If equilibrium concentration of zinc-65 was realized in L. cephalus in water containing 10^{-4} μCi/ml of zinc-65 a potential health hazard would exist for persons continuously consuming more than 4 g per day of contaminated fish flesh. Measurement of radioactivity in L. cephalus taken from an area near the Big Rock Nuclear Reactor revealed that present concentrations of radionuclides in L. cephalus were well below hazardous levels.

PONTOPOREIA AFFINIS (CRUSTACEA, AMPHIPODA) AS A MONITOR OF RADIOACTIVITY IN LAKE MICHIGAN

by

Charles Clifton Kidd Sr.

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy in

(Environmental Health)

The University of Michigan

1970

Doctoral Committee:

Professor G. Hoyt Whipple, Chairman

Professor John C. Ayers

Doctor Claire L. Schelske

Doctor Michael E. Bender

ACKNOWLEDGEMENTS

The growth of the nuclear power industry and the advent of other peaceful uses of atomic energy require that physical scientists whose primary concern is radiation protection become cognizant of the fundamental biological processes at work in our environment. The investigation described in this dissertation involved the study of the reconcentration of radionuclides by an aquatic organism, *Pontoporeia affinis*. The knowledge of aquatic biology and experience in field sampling gained by the writer working aboard the Research Vessel Mysis and the Research Vessel Inland Seas and in the laboratories of the Great Lakes Research Division of The University of Michigan was invaluable. For their aid and continued co-operation I wish to express my thanks and appreciation to the division's director, Dr. David C. Chandler, its scientists and laboratory staff, and crewmen of The Mysis and The Inland Seas.

The chairman and members of my doctoral committee have been of great assistance to me in the preparation of this dissertation.

I would like to express my special thanks to my wife Mary who in addition to her normal duties typed the first draft of the thesis. A special expression of appreciation is due the younger members of my family Charles, Crayton, Chinyere and Cy all of whom have been cooperative and patient.

TABLE OF CONTENTS

Chapter | Page

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I

INTRODUCTION

At this writing a single installation, the Big Rock Point Nuclear Reactor, discharges liquid wastes containing small quantities of radioactivity into Lake Michigan. This power plant produces 240 megawatts (thermal). It is expected that seven nuclear power plants situated on the shores of Lake Michigan will be operating by 1975, and will produce 13,000 megawatts (Bennett 1968). The quantities of radioactive elements discharged to Lake Michigan waters will increase significantly. There is little doubt that the release rates from the facilities will be such that the concentration of any radioactive element in Lake Michigan water will not exceed that maximum permissible concentration (MPC) recommended for the population at large by the National Committee on Radiation Protection (NCRP).[1] However, the fate of these radionuclides in the aquatic ecosystem and their possible radiological health significance resulting from physical and biological processes are not known. It is not acceptable that environmental scientists adopt a wait-and-see attitude. Such scientists are charged with the two-fold responsibility to protect man and the environment from harm due to radiation exposure. Therefore, environmental scientists are frequently engaged in experimentation which is intended to be predictive. Such is the nature of this investigation

---

[1] National Bureau of Standards Handbook 69, U. S. Dept. of Commerce, 1959.

which involves laboratory measurements of accumulated radionuclides in <u>Pontoporeia affinis</u>, a benthic amphipod native to Lake Michigan. The accumulation of a radionuclide results from its uptake, i.e. entry into an organism. Accumulation equals the difference between intake and loss, processes which occur simultaneously.

Often the accumulation of radionuclides by aquatic organisms has revealed to investigators the existence of radiological health hazards. The accumulation of zinc-65 in oyster flesh reported by Preston (1967) is illustrative of this use of biological information. Merlini (1967) described the accumulation of manganese-54 by freshwater clams, and demonstrated their usefulness as indicators of environmental levels of this radionuclide. The present investigation answers the following ecologically important questions:

1. Is <u>Pontoporeia affinis</u> a suitable monitor organism for radionuclides which may be released to Lake Michigan in liquid wastes from nuclear facilities?
2. Does <u>Pontoporeia affinis</u> as a link in the food chain involving man constitute a potential radiological health hazard?

A suitable monitor of radionuclides released to Lake Michigan must be:

1. an organism that lives in Lake Michigan or one that can live there;
2. able to accumulate a radionuclide to such a degree that the mass of an economically obtainable sample will contain at least that quantity of the radionuclide in a 3.5 liter sample of lake water;[1]

---

[1]U. S. Public Health Service Publication N-999-RH-27, Section 5.

3. restricted by habit or physical barriers to migrations within a 10 meter radius of its point of origin; and
4. able to attain 90% of the equilibrium concentrations of accumulated radionuclides in approximately 1 month or less.

It is desirable that the life of a monitor organism be at least 1 year in order that it reflect seasonal variations in environmental levels of radionuclides.

The extent of accumulation of a radionuclide by an aquatic organism is most often characterized by a concentration factor, CF, which relates the concentrations of a radionuclide in the organism and in the surrounding water in the following way:

$$CF = \frac{\text{Radioactivity per gram wet weight of tissue}}{\text{Radioactivity per milliliter of water}} \quad (1)$$

Using common units of radioactivity this ratio is:

$$CF = \frac{\mu\text{Ci per gram wet weight of tissue}}{\mu\text{Ci per milliliter of water}} \quad (2)$$

Where 1 μCi (1 microcurie) of radioactivity is the equivalent of 3.7×10^4 disintegrations per second. The CF represents the ratio of concentrations that are established at equilibrium, steady-state, conditions, i.e., the condition where the processes of intake and loss of a chemical element by an organism proceed simultaneously at the same rate. This situation is never realized. However, in this study naturally occurring concentrations of chemical elements in aquatic organisms and lake water were considered quasi-equilibrium levels, i.e., representative of true equilibrium. Also in the present study, ratios of radionuclide concentrations established between aquatic animals and water as the result of short-term (hours-days) exposures are referred to as

accumulation multiples. Chemical form is a determining factor when considering the availability of radionuclides to an aquatic organism. In the experiments to be described it is assumed that the ionic radioactive species used followed the same biochemical pathways as would dissolved radionuclides in the high purity low-level radioactive wastes released to Lake Michigan by the proposed nuclear facilities.

Ayers (1962) has shown that the total dissolved solids in Lake Michigan waters had increased by 27% over the 50 year period from 1905-1955 due to natural aging of the lake and the introduction of domestic and industrial wastes. Chemical changes of the same magnitude are apparent when one compares concentrations of ionic species in Lake Michigan water analyzed in 1907 and samples analyzed during the present study (see Appendix A). These changes, however, are not rapid enough to invalidate the notions surrounding the use of concentration factors cited above.

The application of equilibrium concentration factors to an aquatic environment containing the maximum permissible concentration of a radionuclide aids in predicting the maximum radionuclide concentrations in local aquatic organisms. Such computations done in the manner of Preston (1967) are very useful in assessing the potential radiological health hazard associated with the consumption of contaminated aquatic organisms. The following general formula can be applied:

$$\text{Maximum permissible consumption} = \frac{(2{,}200 \text{ ml/day}) \text{ X MPC}}{C_f} \qquad (3)$$

In equation (3) 2,200 ml/day is the average individual water intake, and CF is the concentration of a radionuclide in the food organism which at equilibrium is equal to the product (CF) (MPC). Written briefly equation (3) is:

$$\text{Maximum permissible consumption} = \frac{(2{,}200)}{\text{CF}} \text{ g/day} \qquad (4)$$

CHAPTER II

LITERATURE REVIEW

A recently discovered species of gammarid amphipod, Anonyx sp., is in many ways similar to Pontoporeia affinis. Anonyx sp., a marine benthic amphipod was the subject of some radioecological investigations. The studies were conducted by Cross, Dean and Osterberg (1969) for the purpose of examining the metabolism of zinc-65 as affected by temperature. The studies also called attention to the potential role of Anonyx sp., in the cycling of radionuclides. The exposure of experimental animals to radioactive seawater was not chronic. It could best be described as subacute since the duration of all accumulation tests was 8 days, and because it was necessary periodically to remove the animals from the water to measure their radioactivity in a gamma-spectrometer. Accumulation of zinc-65 and its subsequent loss in uncontaminated seawater were measured at 3, 7 and 12° C. Accumulation and elimination rates for zinc-65 by Anonyx sp., were more rapid at the higher temperatures. The presence of sediment during elimination tests was observed to increase the elimination rate. This effect was attributed to the adsorption of released radionuclides by sediment particles preventing thereby any secondary sorption by Anonyx sp. The time required for starved Anonyx sp. to eliminate half of its body burden of radioactive zinc accumulated during the 8-day accumulation tests was 104 days. This rate was reduced to 34 days by feeding Anonyx uncontaminated brine shrimp. The transfer efficiency, i.e. the percentage of

radioactivity in the food of Anonyx sp. that was subsequently accumulated by the amphipods, was observed to be 56% when feeding radioactive brine shrimp. This factor and the elimination times mentioned above are dependent upon kind of food and the feeding rate. Molting was responsible for the loss of 20% of the body burden when radioactive zinc was accumulated from water. Losses of zinc-65 activity were reduced to 2% of the body burden when food was the sole source of the radioactive zinc. Cross, et al. (1969) postulated that for Anonyx sp. food was more important than water as a source of radionuclides. The localization of radioactivity on the surface of Anonyx sp. and its subsequent loss through molting severely limited the usefulness of zinc-65 as an indicator of metabolic processes. These same considerations suggest that Anonyx sp. transfer significant quantities of radioactivity from radioactive plumes to bottom sediments. Cross, et al. also suggested that zinc-65 in the flesh of peamouth chubs (Mylocheilus caurinum) resulted from this fish's consumption of zinc-65 contaminated Anonyx sp.

Fowler, Small and Dean (1969) conducted laboratory experiments to determine if trace concentrations of zinc-65 could be used to measure metabolism in the ecologically important pelagic marine crustacean, Euphausia pacifica. It was also the objective of these investigators to gain some insight into the role of euphausiids in the transport of large concentrations of zinc-65. Accumulation of zinc by live euphausiids was studied at 5, 10 and 15° C over a period of 10 days. The same test animals were allowed to eliminate zinc-65 in uncontaminated environments. Both accumulation and elimination processes were affected by the molting of euphausiids. Molted exoskeletons averaged 41% of the total body burden of zinc-65. It was observed that concentrations of zinc-65 in euphausiids were inversely proportional to animal size.

The fact was also reported that the Q_{10}, ratio of the reaction rates at 5 and 15° C, for zinc-65 uptake by Euphausia pacifica was nearly twice that for oxygen consumption. The author suggests that the process of zinc-65 accumulation in Euphausia pacifica may not be a biological one. The fact that the amounts of zinc-65 accumulated in 10 days by living and dead Euphausia pacifica are not significantly different is cited as further evidence of the physical nature of zinc-65 accumulation by these amphipods. The authors conclude that zinc-65 cannot be used as an index of metabolism in Euphausia pacifica, and therefore that these animals cannot serve as monitors of environmental zinc-65 concentrations. It should be pointed out that the duration of the accumulation experiments with Euphausia pacifica, 10 days, was short compared to its life expectancy, 2 years, and that the animals were not fed radioactive food in any of the experiments. These facts probably account for the large proportion of adsorbed zinc-65 in live Euphausia pacifica used in these experiments.

The zinc-65 transfer capability of Euphausia pacifica was investigated thoroughly by L. F. Small (1969). His investigation revealed that the transfer of zinc-65 from floating lenses of radioactive seawater to non-radioactive near-bottom seawater by Euphausia pacifica was significant. It was shown that maximum body burdens of zinc-65 in Euphausia pacifica were realized in 5 days in radioactive seawater at 10° C. These body burdens varied from 2 μCi/g (wet weight) to 5 μCi/g depending upon the period of time (8 to 16 hours) that the amphipods spent in the radioactive seawater pool which contained 25 μCi/l. When this radioactive seawater was the sole source of zinc-65 the amphipods lost an average of 25% of their body burden in cast exoskeletons.

Equilibrium body burdens of zinc-65 in *Euphausia pacifica* were not realized during 5-day periods when the amphipods were allowed to graze phytoplankton (initial concentration 243 X 10^3 cells/ml) labeled with 25 μCi/l. Molting was responsible for the 10% loss of the 5-day body burden of zinc-65 in *Euphausia pacifica* used in these experiments. Under these same conditions fecal deposition was responsible for the loss of 41% of the initial body burden.

Alley (1968) and Marzolf (1968) in separate studies demonstrated the apparent preference of *Pontoporeia affinis* for organic sediments. Moreover, Marzolf demonstrated a direct relationship between bacterial populations in the sediment and population densities of *Pontoporeia affinis*. No optimum value of carbon content of the sediment was specified in either investigation. In large scale field investigations by J. Lellak (1965) there were no correlations between population densities of bottom fauna and carbon content of the mud in several experimental ponds. Lellak demonstrated in a unique experiment that after sterilization and devegetation of several ponds the last community to revitalize itself was the benthic community. This demonstrated the dependence of bottom fauna on food supply from the overlying water column transported to the bottom by fecal material and expired organisms. These results also suggested that bacteria may be a source of energy for benthic amphipods. W. Wisen (1956) has observed that the burrowing malacostracan *Cumella vulgaris* feeds by grazing bacteria and algae from the surfaces of soil particles. Studies by Hargrave (1970) revealed that a burrowing fresh water amphipod *Hyallella azteca* ingests algae and bacteria from the surfaces of detrital particles.

CHAPTER III

MATERIALS

A. Experimental Animals

Pontoporeia affinis (Lindstrom) (Figure 1) is a small burrowing amphipod (sideswimmer, scud) which belongs to the Subclass, Malacostraca of The Class Crustacea. One of the relatively few freshwater amphipods, it is believed to be a glacial relic which has adapted to the low salinity of the Baltic and Great Lakes. (Pennak 1953) It is found in Lake Michigan at depths of from 10 to 270 meters, and bottom temperatures from 2° C to 18° C. (Alley, 1968) *Pontoporeia affinis* is thought to be an omnivorous species which consumes larval forms of aquatic insects, diatoms, and organic debris including its own exoskeleton which it periodically sheds during maturation (Pennak 1953). It is in fact a semi-pelagic species, i.e. it carries on periodic vertical migrations (Wells 1960). The life-span of *Pontoporeia* at the temperatures and depths found in Lake Michigan varies from 2 to 3 years (Alley 1968). The female of this species maintains the newly hatched young in an abdominal marsupium or brood-sac for approximately one week. At the end of this time the female gives birth to free-swimming young. Reproduction usually takes place between October and March (Alley 1968).

During the spring and summer of 1967 the writer worked with biologists of The University of Michigan Great Lakes Research Division aboard the organization's research ships, Mysis and Inland Seas. The

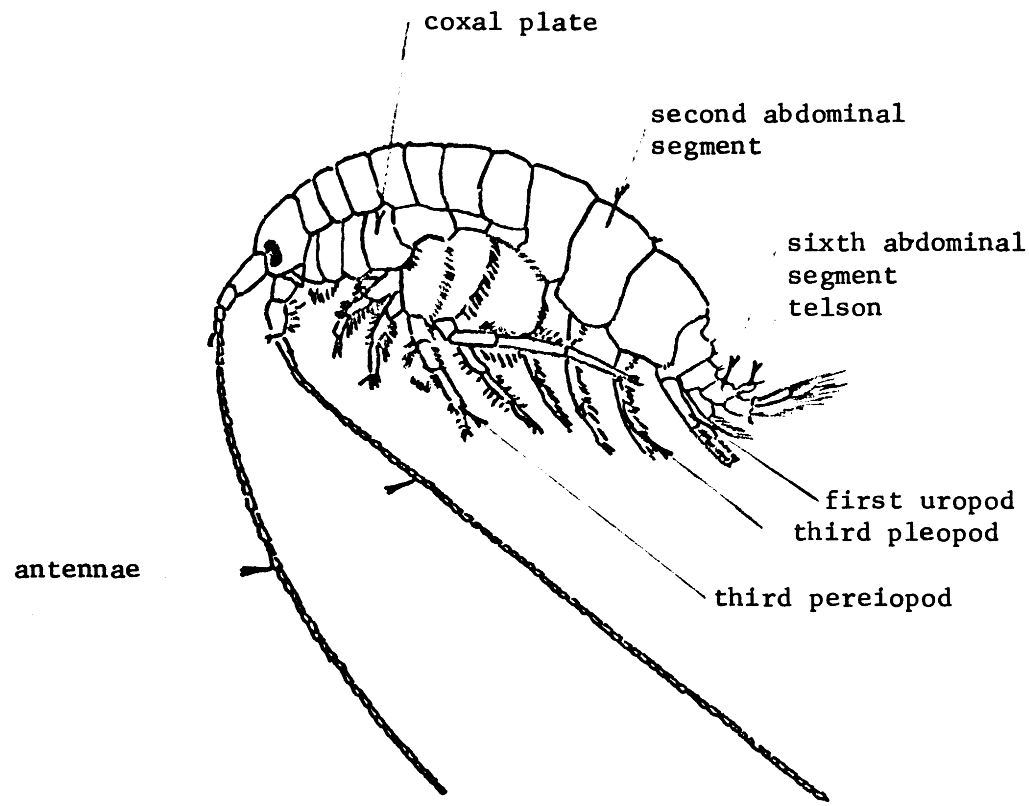

Fig. 1. Morphological features of Pontoporeia affinis.
Mature male, 9X actual size (approximate wet weight = 5mg)

work provided first hand experience in the routine sampling of lake water, sediment, planktonic organisms and benthic organisms. It was my observation that of the communities routinely surveyed for ecological considerations the benthic community would probably yield a suitable biological indicator of radioactivity especially with regard to the criteria of year-round abundance and localization. Of the three dominant species in the benthic community, the amphipod, Pontoporeia affinis, oligochaetes (benthic worms), and sphaeriids (fingernail clams) Eggleton (1937) found Pontoporeia affinis to be most abundant. More recently Powers and Alley (1967) reported that the average standing crops corresponded to 13 g, 6 g, and 1 g (wet weight of organisms per square meter) for Pontoporeia, oligochaetes and sphaeriids respectively. These figures reflect the continued dominance of Pontoporeia. The distribution of Pontoporeia affinis in Lake Michigan is shown in Figure 2. Because Pontoporeia affinis was the most widely distributed and the most abundant benthic organism in Lake Michigan it was chosen for further evaluation as to its usefulness as a monitor of environmental radioactivity.

B. Radiation Detection

1. Gamma-ray counting system

Gamma-radioactivity in water and Pontoporeia used in the laboratory experiments to be described was measured with a gamma-ray scintillation spectrometer. This spectrometer was composed of a 12.5 X 12.5 cm NaI (Tl) crystal connected to a 100-channel multichannel analyser (MCA). The crystal was housed in a lead cylinder (inside dia. = 18 cm) which provided 6 cm of shielding on all sides. Operating voltage for this system was maintained at 900 volts. The discriminator level and

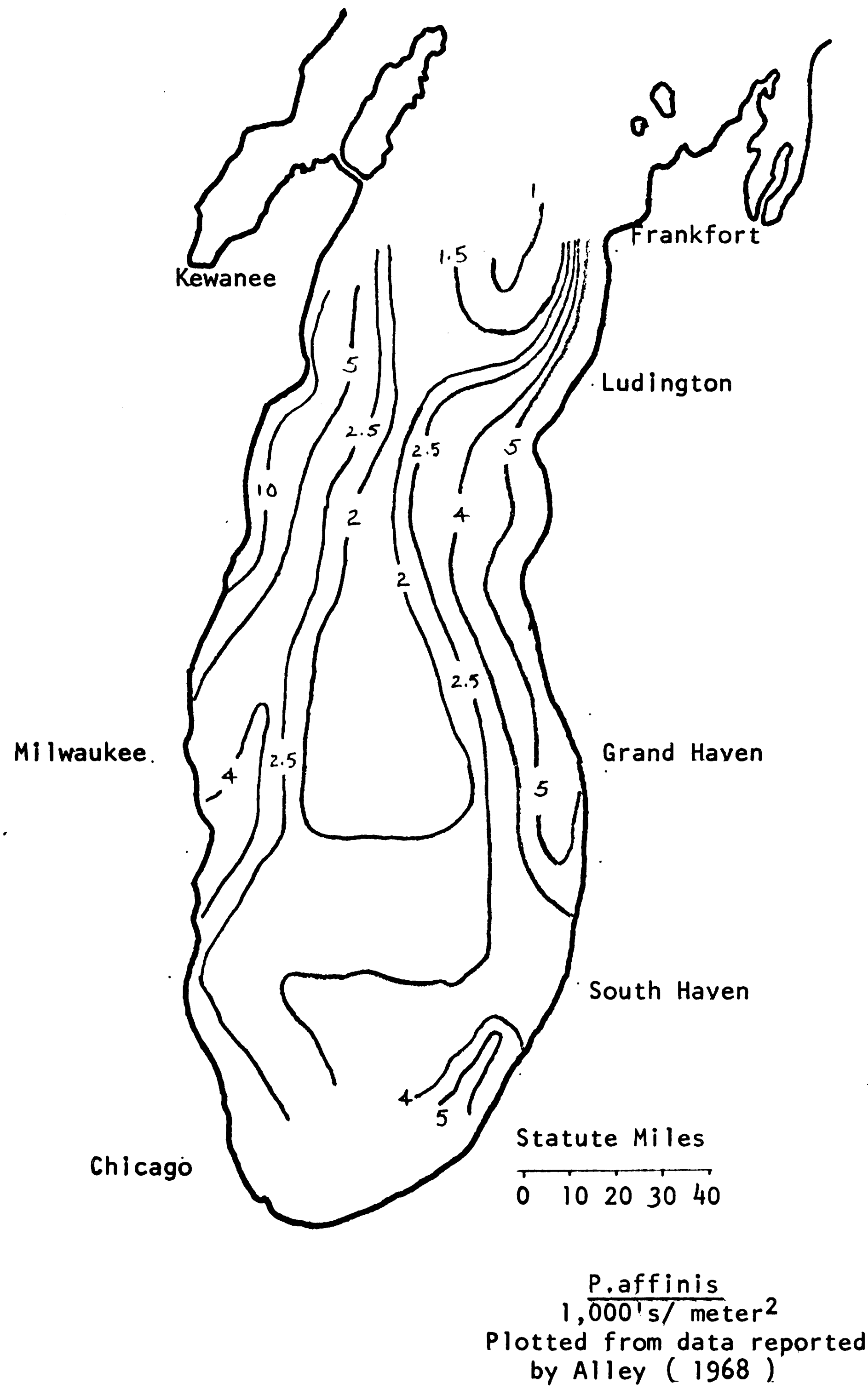

FIGURE 2. DISTRIBUTION OF PONTOPOREIA AFFINIS IN LAKE MICHIGAN

conversion gain controls for the MCA were adjusted so that linear relationships existed between gamma photon energies and gamma spectrum channel numbers, and between gamma photon energies and gamma detection efficiencies (counts per gamma). Measurement of background radiation revealed the counting rates shown in Table 1.

2. Natural levels of radioactivity in water and *Pontoporeia*

During the time when laboratory experiments were being performed several samples of lake water and *Pontoporeia affinis* from the Grand Haven, Michigan sampling area (Figure 3) were analyzed for gamma radioactivity. The results of those analyses are shown in Table 1.

TABLE 1

BACKGROUND RADIOACTIVITY AND RADIOACTIVITY IN LAKE WATER AND *PONTOPOREIA AFFINIS*

Sample	Energy Bands[1]	Count Rate, cpm	Counting Time, Min.	Net Count, cpm ± 0.95 Error
background	.42- .60 Mev	289	100	0 ± 3.4
	.76- .92 Mev	211	100	0 ± 2.8
	1.02-1.18 Mev	153	100	0 ± 2.4
Pontoporeia affinis, 4.25 g	.42- .60 Mev	298	100	9.0 ± 4.8
	.76- .92 Mev	210	100	0 ± 4.2
	1.02-1.18 Mev	157	100	4.0 ± 3.5
Lake Michigan water, 4,000 ml	.42- .60 Mev	296	100	7.0 ± 4.8
	.76- .92 Mev	221	100	10 ± 4.2
	1.02-1.18 Mev	167	100	14 ± 3.5

[1]Include the principle photo peaks of Sr-85, Mn-54 and Zn-65 respectively.

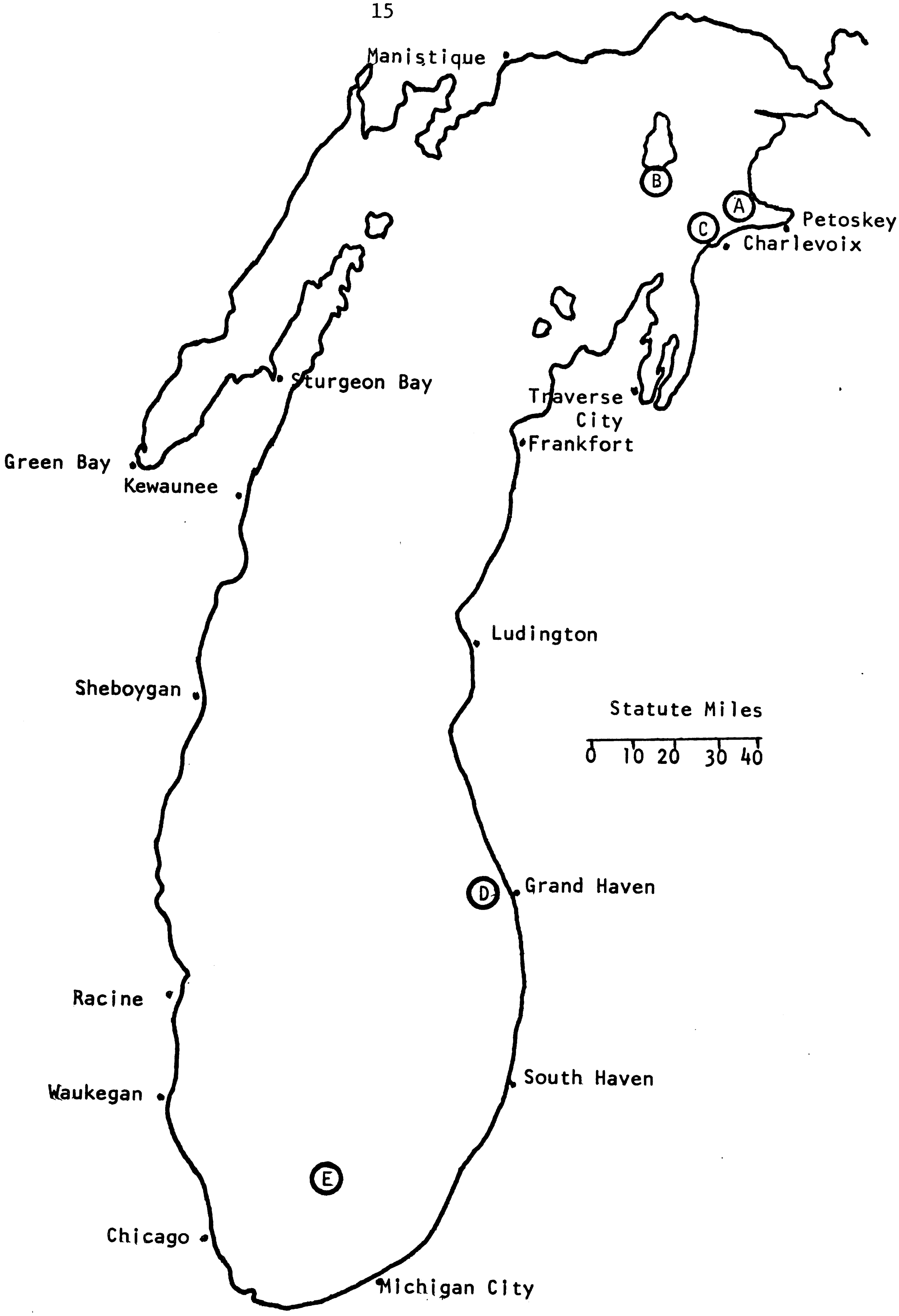

FIGURE 3. PONTOPOREIA SAMPLING POINTS IN LAKE MICHIGAN

These levels of gamma radioactivity indicate that little error would be introduced by natural radioactivity in the determination of radioactivity in 10 ml samples of contaminated lake water or 100 mg samples of contaminated Pontoporeia affinis used in laboratory experiments.

3. Minimum detectable counting rates

Associated with the net counting rate of a sample is a certain amount of random error which can be computed in accordance with the following equation (Pelletier 1966):

$$E_z = Z\left(\frac{N_x + N_b}{t_s} + \frac{N_b}{t_b}\right)^{1/2} \qquad (5)$$

Where: E_z = error (cpm)

Z = constant associated with a given confidence level

N_x = net counting rate of sample (cpm)

N_b = background counting rate (cpm)

t_s = sample counting time

t_b = background counting time

The error can also be expressed as a fraction of the net counting rate N_x, and is given by this expression:

$$\text{Fractional Error} = FC_z = E_z/N_x \qquad (6)$$

It was considered essential that the net counting rate of a sample equal or exceed error corresponding to the 99% level of confidence. The

fractional error for the minimun detectable net counting rate, Nx (minimun), is numerically equal to 1.

In the absence of significant levels of radioactivity in clean lake water and Pontoporeia affinis the background counting rates of Table 1 are used to compute the random error. Samples were counted for 20 minutes.

Substitution in the manner of Pelletier (1966) into equation (5) will lead to the following expression for the minimum detectable count rate:

$$N_x \text{ (minimum)} = \frac{Z^2}{2t_s(FC_z)^2}\left\{1 + \left[1 + \frac{4N_b FC_z^2 t_s(t_b + t_s)}{Z^2 t_b}\right]^{1/2}\right\} \quad (7)$$

At the 99% confidence level: $Z = 2.58$

$FC_z = 1$

$t_s = 20$ min.

$t_b = 100$ min.

Therefore:

$$N_x \text{ (minimum)} = \frac{(2.58)^2}{40}\left\{1 + \left[1 + \frac{9{,}600\ N_b}{640}\right]^{1/2}\right\} \quad (8)$$

$$= 0.16 + 0.16\ (1 + 15\ N_b)^{1/2} \quad (8)$$

Evaluation of this expression over energy band widths corresponding to the position of the principal absorption peaks of strontium-85, manganese-54, and zinc-65 (background activities in Table 1) revealed that the minimum detectable counting rates were 11.0 cpm, 9.0 cpm and 8.0 cpm in these areas respectively.

4. Counting efficiencies

Ten ml samples of experimental waters and samples of live Pontoporeia affinis in 10 ml of clean lake water were put into plastic evaporating dishes which measured 6 X 6 X 2.5 cm. In this configuration the samples were placed on the NaI crystal at its center and counted for 20 minutes.

In order to determine counting efficiencies and to test the linearity of the detection system's response to gamma-rays, standard solutions of radionuclides were dispersed in 10 ml of distilled water in plastic evaporating dishes. These standards were counted in the same position on the detector face. The carrier-free standard solutions were supplied by Nuclear Science and Engineering Corporation, Pittsburgh, Pa. on February 19, 1969. The original 1 μCi isotopic solutions were dispersed in 6*N* HCl. The counting efficiencies determined by this method are shown in Table 2. Figure 4 shows the linearity (counts/gamma vs. gamma energy) of the scintillation detection system.

The graph in Figure 4 also shows the results of a typical performance check of the counting system. One-year-old thimble shaped spectrometer standards were used to make these tests. These sources were supplied by Tracerlab-Inc., and were calibrated by the manufacturers to ± 5% of the stated gamma-ray intensity. The thimble sources were placed at the center of the crystal standing on their flat ends and counted. The difference in counting geometries account for the observed differences in liquid standard and thimble source counting efficiencies.

TABLE 2

GAMMA-RAY COUNTING EFFICIENCIES

	Standard in 10 ml H_2O	Gamma Abundance	Net cpm in Peak Area	Peak Efficiencies (Counts/gamma) X 100
^{85}Sr	2.73 μCi (6.06 X 10^6 dpm)	0.51 Mev, 100%	1.62 X 10^6	26.6%
^{54}Mn	4.43 μCi (9.83 X 10^6 dpm)	0.84 Mev, 100%	2.14 X 10^6	21.8%
^{65}Zn	1.84 μCi (4.07 X 10^6 dpm)	1.14 Mev, 44%	0.31 X 10^6	17.4%
^{40}K*	0.004 μCi (9,650 dpm)	1.46 Mev, 11%	113	10.7%

*^{40}K standard composed of 10 ml solution of KCl containing 5.10 g stable potassium.

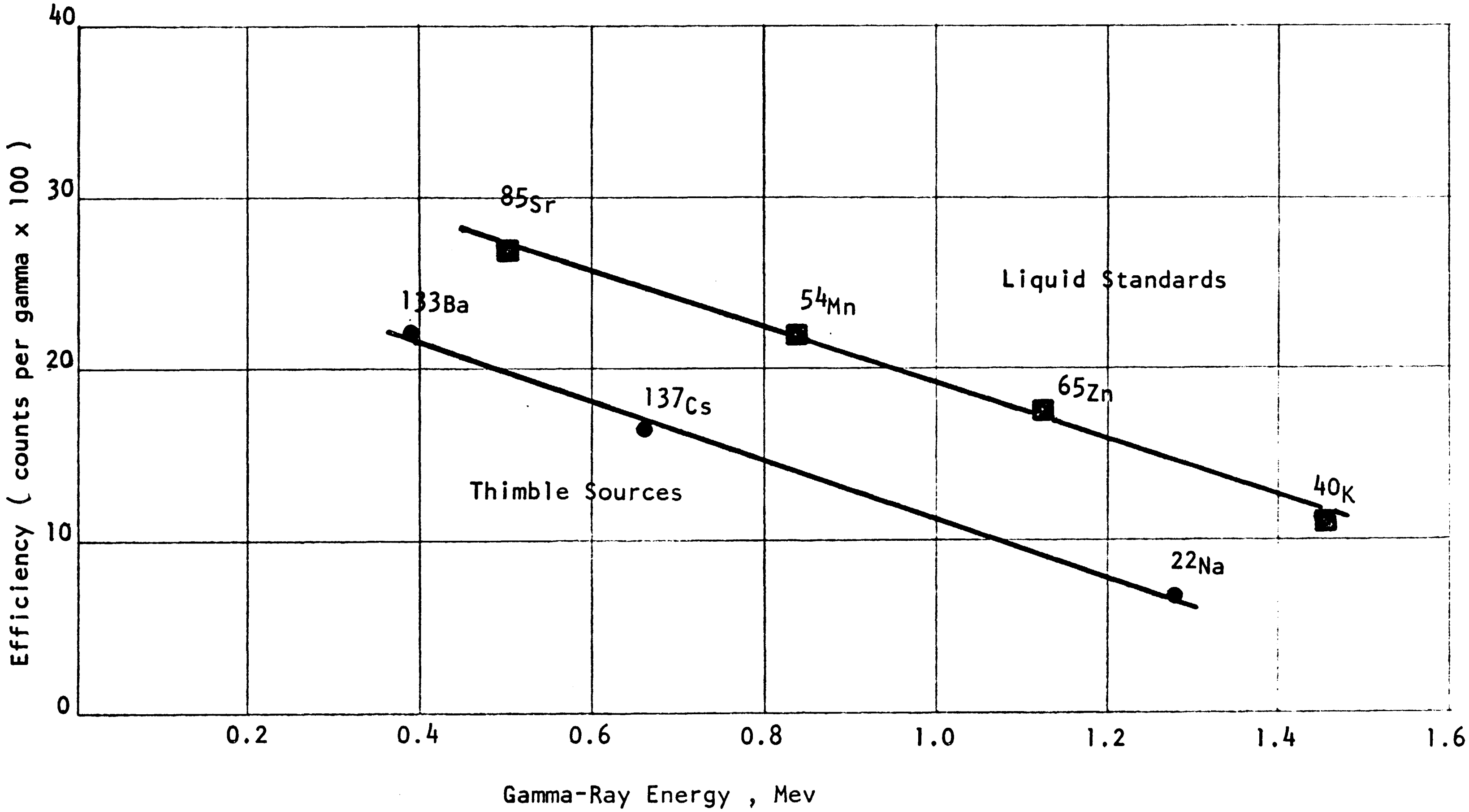

FIGURE 4. EFFICIENCY vs. GAMMA-RAY ENERGY

5. Minimum detectable radioactivity

The minimum amount of radioactivity due to strontium-85, manganese-54 and zinc-65 that can be reliably measured by the scintillation detection system and sample configuration already described can be calculated by dividing the minimum detectable counting rates stated above (para. 3) by the appropriate counting efficiency and gamma abundance from Table 2. For example:

$$\text{Minimum detectable strontium-85 activity in samples} = \frac{(11\ \text{cpm})}{(3.7 \times 10^{4}\ \frac{\text{dps}}{\mu\text{Ci}})(60\ \frac{\text{sec}}{\text{min}})(0.266\ \frac{\text{cpm}}{\gamma\text{pm}})(1.0\ \frac{\gamma\text{pm}}{\text{dpm}})}$$

$$= 1.86 \times 10^{-5}\ \mu\text{Ci}$$

Similar calculations reveal minimum detectable levels of manganese-54 and zinc-65 activities to be 1.86×10^{-5} μCi (the same as for strontium-85) and 4.67×10^{-5} μCi respectively.

6. Interference corrections in compound spectra

In some extended laboratory experiments it was advantageous to utilize fully the capability of the MCA system to measure gamma-ray intensities from several sources simultaneously. In some of the experiments to be described strontium-85, manganese-54 and zinc-65 were all present in individual samples. A set of simultaneous equations is developed in Appendix B to correct for the mutual interference of these sources.

CHAPTER IV

EXPERIMENTAL METHODS

A. Sampling Techniques

Live specimens of Pontoporeia affinis were taken with a Ponar Grab Sampler (Figure 5) from a position on the bottom of Lake Michigan three miles west of the pier at Grand Haven, Michigan (pt. D, Figure 3). The sampling area lay approximately 36 meters below the water surface. Live P. affinis in mud and water from the sampling point were transported to the biology laboratory of The Great Lakes Research Division at Ann Arbor, Michigan. During the 2 1/2 hour trip by automobile the amphipods and their mud-water environments were maintained at 8° C - 10° C in a large plastic lined, steel jacketed ice chest to which copious quantities of ice were added in the form of the frozen contents of sealed 1-liter polyethylene bottles. All plastic in contact with the amphipods or their environment was of the non-toxic variety commonly used in biology laboratories. The danger of loss of organisms due oxygen depletion was negligible because of the low temperature and because these amphipods thrive at oxygen tensions as low as 7% saturation at 10° C (Segerstrale 1959). Live specimens of P. affinis for all laboratory experiments were taken from their habitat and transported to the laboratory in this manner.

FIGURE 5. PONAR DREDGE SAMPLER IN CLOSED CONFIGURATION.

B. Identification of Radionuclides Accumulated by Pontoporeia affinis

1. Nuclear facilities wastes (Experiment-1)

Approximately 100-150 amphipods were placed in each of two 500 ml polyethylene aquaria. Each aquarium also contained 300 ml of lake water and 30 g of sediment. Samples of liquid wastes were obtained from the Big Rock Nuclear Power Plant and The Nuclear Fuel Service Reprocessing Plant at West Valley, New York and were analyzed with the gamma-ray counting system. Three ml of each of these wastes were added to the two aquaria (one waste to each aquarium). The amphipods in their contaminated environments were maintained at 10° C in darkness for 24 hours. At that time some of the amphipods were removed by disturbing the aquarium and catching the amphipods in a tea strainer. Three groups of 30 to 50 amphipods were taken from each of the two aquaria. The amphipods were weighed and assayed for gamma radioactivity in 10 ml of uncontaminated lake water. The analysis for gamma emitting radionuclides was made by placing the exposed amphipods and lake water in flat plastic evaporating dishes on the NaI(Tl) scintillation crystal previously described. This brief experiment was done in order to identify the radioactive isotopes which the amphipod could accumulate.

2. Laboratory solutions - (Experiment-2)

In order to confirm and accurately quantify the results of the first brief identification experiments Pontoporeia affinis were exposed to mud-water environments each containing one of seven radioactive elements in solution, cerium-144, manganese-54, zinc-65, cesium-137, zirconium-95, ruthenium-106, and strontium-90. These radionuclides are

frequently found in wastes from nuclear facilities. Each three ml volume of solution containing a radionuclide was analyzed for gamma radioactivity and added to a separate plastic aquarium containing 30 to 40 amphipods, 250 ml of lake water and 30 g of sediment. All aquaria were maintained at 10° C for 72 hours. During this time the water in each covered plastic aquarium was continuously aerated. Amphipods from these tests were divided into three groups, weighed and counted. Direct analysis of P. affinis for gamma-emitting radionuclides was made using the NaI (Tl)-multichannel analyzer system. P. affinis from the strontium-90 test were wet-digested with nitric acid and the dry residue was counted for 100 minutes in a Beckman Low-Background Beta Counter. The gamma and beta activities remaining in the waters from the test aquaria were determined. The background activities for the energy span used to estimate gamma-emitter activities, and the background beta activity were subtracted. The net activities of each of the radionuclides accumulated in the amphipods permitted the calculation of accumulation multiples.

C. Measurement of Stable Element Concentrations in Lake Water, Pontoporeia affinis and Fish Flesh (Experiment-3)

In order to determine the equilibrium concentration factors for strontium, manganese and zinc in P. affinis it was necessary to measure the concentrations of these stable elements in samples of lake water and P. affinis. To evaluate the potential radiological health hazard to man posed by the accumulation of radionuclides in Pontoporeia it was necessary to analyze samples of food-fish known to subsist predominantly on P. affinis (Schmitt 1965). The flesh of chubs (Leuciscus cephalus) was analyzed for stable strontium, manganese and zinc. Table 3 gives the

location of all environmental sampling stations. All chemical analyses were made using atomic absorption spectrophotometry.

TABLE 3

LOCATION OF ENVIRONMENTAL SAMPLES

Sample	Location	Date
Water	S. Lake Michigan, pt. D of Figure 3	March and July, 1969
Pontoporeia affinis	N. Lake Michigan, pts. B&C Figure 3	May, 1969
Pontoporeia affinis	S. Lake Michigan, pt. D, Figure 3	October, 1968
L. cephalus	N. Lake Michigan, pt. A, Figure 3	October, 1968

P. affinis and *L. cephalus* were digested in 6*N* HNO_3, and diluted to the desired volume with dionized water. Three 4 liter samples of filtered Lake Michigan water were slowly evaporated over a steam bath until each was reduced to 20 ml. Two ml of concentrated HNO_3 was added initially to each sample to minimize the loss of chemical elements due to plating on the walls of the evaporating vessel.

Analyses for manganese and zinc were made on a Jarrell-Ash atomic absorption unit. Strontium analyses were made using a Perkin-Elmer model 290 atomic absorption unit. Acetylene and air was the fuel mixture used in the analyses for zinc and manganese. Monochromatic light sources having wave lengths of 2139 A (angstroms) and 2795 A were used in the analyses of zinc and manganese respectively. A mixture of acetylene and

and nitrous oxide was the fuel used in strontium analyses, and the wave length of the light source was 4607 A.

Samples of L. cephalus were analyzed for gamma-emitting radionuclides using the gamma-ray counting system. Samples of HNO_3 and deionized water were used as blanks. Samples were prepared for strontium analysis by increasing their sodium concentration to avoid the ionization of stable strontium atoms in the flame. Previous analyses had demonstrated that the addition of enough NaCl to effect a sodium concentration of 2,000 ppm was sufficient to prevent errors from this source.[1] The amount of strontium in NaCl used was not determined.

D. Measurement of the accumulation and elimination of strontium-85, manganese-54 and zinc-65 by P. affinis

1. Accumulation of radionuclides (Experiment-4)

Three large polyethylene aquaria were filled with 2 liters of lake water and 1,000 g of fresh sediment from the sampling area shown in Figure 3 (pt. D). Water in each aquarium contained 0.2 μCi each of radioactive strontium-85, manganese-54 and zinc-65. Each initial concentration was equal to 10^{-4} μCi/ml (NCRP recommended MPC). They are, therefore, the concentrations acceptable individually in public drinking water. All isotopes were added to the lake water in the carrier-free state. Each of the aquaria then received approximately 500 P. affinis of similar size, 5 mg each (6.0-9.5 mm in length). Each aquarium thus prepared was continuously aerated and maintained at 10° C in darkness for 24 hours. At the end of the 24 hour period a sample of water (25 ml) and between 20 and 30 P. affinis were removed from each aquarium and counted with the

---

[1] R. Rossman, Great Lakes Research Division, University of Michigan.

100-channel NaI (Tl) multichannel analyzer system previously described. All water was drained from the aquaria. The aquarium and draining device used is shown in the drawing in Figure 6. Each aquarium and draining device used is shown in the drawing in Figure 6. Each aquarium was carefully refilled with 2-liters of fresh lake water containing the same initial concentrations of strontium-85, manganese-54 and zinc-65 (1 X 10^{-4} µCi/ml). Counted amphipods were returned to their original aquaria and exposure was continued for another 24 hour period. The weighing and gamma counting of amphipods and refilling of aquaria required 30 minutes or less during which the amphipods remained in the 10 ml of lake water in which they were counted while living. Radioactive water and radioactive amphipods in contaminant-free lake water were counted in thin polyethylene evaporating dishes. Exposure and daily radioassay of <u>P. affinis</u> were continued until no further increase in concentration was apparent or until 10% of the test animals died from repeated handling. The simultaneously observed strontium-85, manganese-54 and zinc-65 activities were corrected for radioactive decay, background radioactivity, and for spectral interference. The radioassay of exposed amphipods and contaminated water permitted the calculation of concentration factors.

The molar concentrations of strontium-85 used was .02 times the molar concentration of strontium-90 permitted in public drinking water, and 0.4 times the allowable molar concentration of strontium-89. Strontium-85 was used because of the convenience with which its gamma emission could be detected and measured.

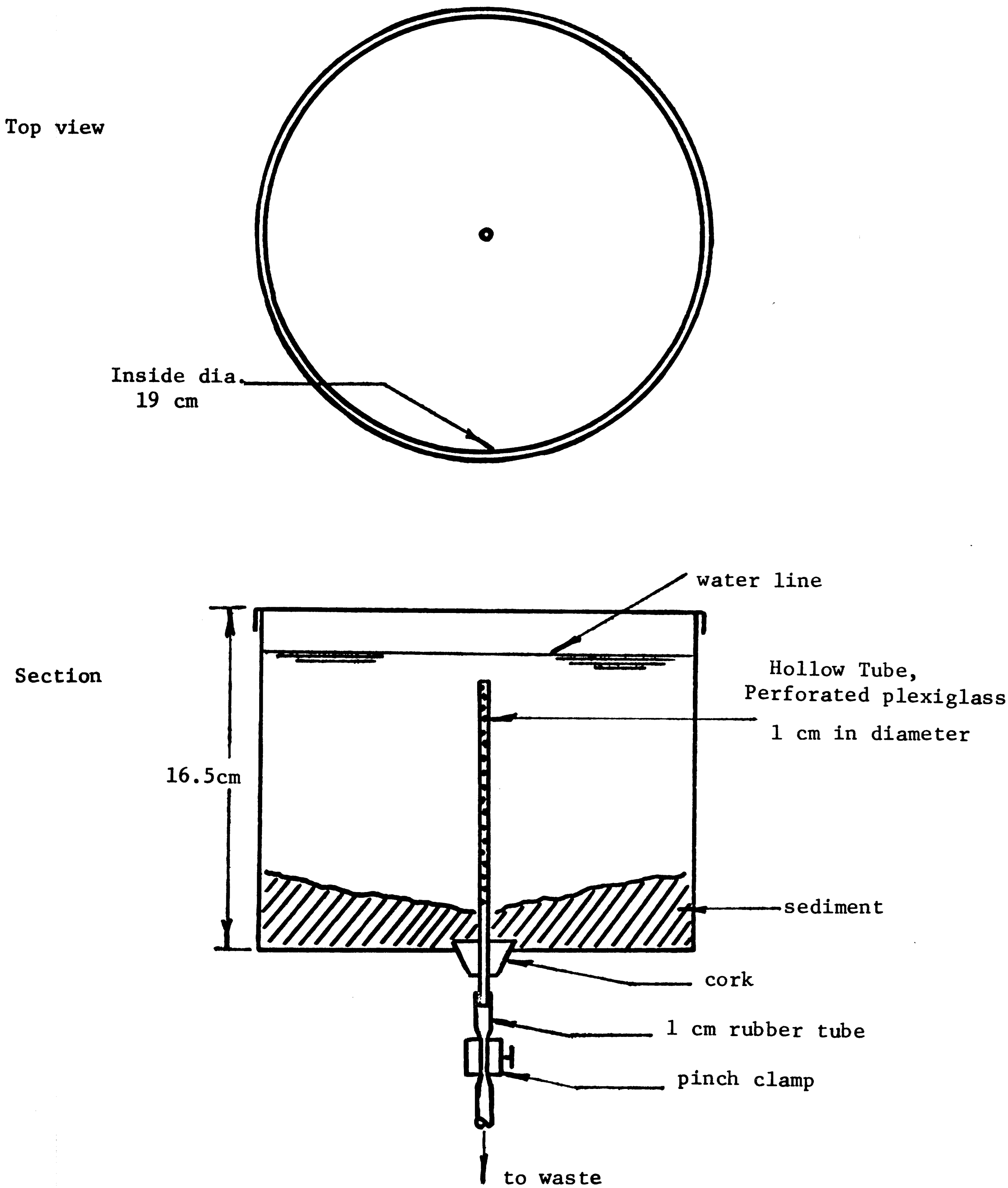

Fig. 6. Large aquarium and draining device.

2. Elimination of Radionuclides (Experiment-5)

Approximately 150 P. affinis were put in a polyethylene aquarium containing sediment and lake water having 17 X 10^{-4} μCi/ml each of strontium-85 and manganese-54 and 8.0 X 10^{-4} μCi/ml of zinc-65. This culture was kept at 10° C for 6 days. The radioactive amphipods were divided into three groups of approximately 50 animals each. Each of these groups was placed in a large aquarium containing clean lake water and sediment. The prepared amphipods and aquaria were maintained for 24 hours in darkness at 10° C. At the end of the 24 hour period the amphipods were removed from each aquaria weighed, and then counted using the gamma-spectrometer. The counted amphipods were put into a second set of aquaria containing clean lake water and mud from the sampling area shown in Figure 3 (pt. D). These newly prepared amphipods were maintained once again at 10° C for 24 hours. Observed concentrations of radioactive elements in test amphipods were corrected for radioactive decay, spectral interference, and background radiation. A plot of the average percentage of remaining activity versus the time the amphipods spent in the clean aquaria allowed the immediate determination of the biological half-life, T_B, of each radionuclide accumulated.

E. Determination of the Distribution of Radionuclides Accumulated by P. affinis

1. Removable and non-removable radioactivity (Experiment-6)

A simple definition of removable radioactivity is that portion of accumulated radioactivity that is superficially associated with an organism. In this experiment the removable radioactivity accumulated by

living P. affinis was defined as the portion of radioactivity removed from freshly killed radioactive amphipods by storing them in non-radioactive preservative solutions. The non-removable radioactivity was therefore defined as the quantity of radioactivity remaining associated with the amphipods after storage.

Three groups of 25-30 P. affinis each were maintained in separate aquaria at 10° C for 72 hours. The aquaria contained sediment and waste water from Experiment-4. The individual concentrations of strontium-85, manganese-54 and zinc-65 were not measured, but due to their origin must have been between 10^{-5} and 10^{-4} μCi/ml. P. affinis from each aquarium were removed with a tea strainer, and placed in an oven at 103° C for 5 minutes. The killed amphipods were counted to establish initial gamma radioactivity. They were then put in solutions containing 10% formaldehyde where they remained for 15 days. At the end of this period the P. affinis were removed, blotted to remove excess liquid and counted in the scintillation detection system. After corrections for radioactive decay, interference and background radiation, the residual levels of radioactivity were subtracted from the initial levels to determine the removable radioactivity due to each radionuclide.

The use of formalin in the soaking solutions prevented bacterial degradation of the amphipods. The experiment was repeated twice using the same number of P. affinis in each sample.

2. Radionuclides in the exoskeleton of P. affinis (Experiment-7)

Approximately 150 amphipods from Experiment-4 described above were divided into groups of 50 amphipods weighed, and radioassayed for gamma radioactivity. Each group of amphipods was then placed in a glass vial

containing 20 ml of Nuclear Chicago Corporation NCS Reagent, a combination of strong basic chemicals normally used to prepare biological materials for scintillation counting. The dead amphipods were maintained at 40° C for approximately 24 hours. Microscopic examination revealed that the soft tissue had been digested leaving the chitinous exoskeletons. The shells were rinsed in toluene, rinsed in water at 100° C, and counted for strontium-85, manganese-54 and zinc-65, radioactivity.

F. Investigation of certain environmental factors which influence radionuclide accumulation by P. affinis

1. Temperature (Experiment-8)

As stated in section A of chapter III P. affinis inhabit areas of Lake Michigan where the temperature may be as low at 2° C or as high as 18° C. To measure the effect of temperature on radionuclide accumulation, experiments were conducted at temperatures of 3° C, 10° C, 13° C and 20° C which span the range of temperatures preferred by the organism, 8-12° C (Segerstrale 1959). Aquaria containing 1 liter of lake water, 1 kg of sediment, equal quantities of strontium-85, manganese-54 and zinc-65, and 200 similar sized amphipods were maintained at each of the temperatures cited above for 12 hours. The rates of accumulation of strontium-85, manganese-54 and zinc-65 by P. affinis at each temperature were measured.

2. pH (Experiment-9)

The pH of the lake water at the sampling point varied between 6.0 and 8.8. At the time of this experiment the lake water taken from the sample area had pH of 7.8. The pH of each of two 1 liter volumes of lake water was adjusted to 9.3 with 3N NaOH. The pH of two other 1 liter

volumes of lake water was adjusted to 5.6 by the addition of 5N HCl. Four aquaria each containing water at an adjusted pH and two additional aquaria each of which contained 1 liter of lake water at pH 7.8 all received 200 similar sized amphipods and equal quantities of strontium-85, manganese-54 and zinc-65. All aquaria were incubated for 12 hours at 10° C. The concentrations of strontium-85, manganese-54 and zinc-65 attained by the amphipods at the different pH levels were measured. The pH of water used was not determined at the conclusion of the experiment.

3. Sediment (Radionuclide Routing, Experiment-10)

The experiment to be described was designed to reveal the influence of bottom sediment on the uptake and accumulation of strontium-85, manganese-54, and zinc-65 by *P. affinis*. The experiment also measured the effect of sediment sterilization on radionuclide accumulation by *P. affinis*.

Fresh sediment from the sampling area shown in Figure 3 (pt. D) was put into the bottom half of a petri dish and heated in an oven at 115° C for 15 minutes. Upon cooling the sample of sterilized sediment was placed in the bottom of a polyethylene aquarium. The aquarium was then filled with lake water containing strontium-85, manganese-54 and zinc-65. Two additional petri dishes containing unsterilized sediment were put into polyethylene aquaria to which radioactive lake water was added. All three aquaria were maintained at 10° C for 12 hours. At the end of this time each sediment-filled petri dish with the exception of one which contained unsterilized sediment was carefully removed from the radioactive water and placed in a clean polyethylene aquarium that was then filled with clean lake water.

The levels of radionuclides remaining in the waters from the altered aquaria were determined by gamma-ray analyses of 25 ml samples of this water. The two aquaria containing radioactive sediment and clean lake water, the unaltered aquarium containing radioactive water and sediment, and an aquarium containing radioactive water only were all seeded with 20 to 30 similar sized P. affinis. All cultures were maintained at 10° C for 12 hours. At the conclusion of the incubation period all amphipods were transfered to aquaria containing clean water and sediment. The radioactive P. affinis remained in these aquaria for 2 hours which was ample time for the amphipods to clear their guts of radioactive material (Johannes 1964). Samples of radioactive waters and P. affinis were analyzed for gamma radioactivity. The entire experimental procedure was repeated twice. The data from these tests were used to calculate the quantities of strontium-85, manganese-54 and zinc-65 present in the sediment, water and P. affinis from each of the laboratory environments.

4. The bottom deposition and decay of radioactive organic matter (Experiment-11)

The experiment to be described here was designed to reveal the relative contribution of dead planktonic organisms to the accumulation of strontium-85, manganese-54 and zinc-65 by P. affinis. The experiment will also measure the effects of decomposition of radioactive organic matter on P. affinis accumulation of these radionuclides.

A laboratory culture of green algae, principally Chlorella pyrenoides, was grown in 2 liters of Lake Michigan water to which 0.01 μCi each of strontium-85, manganese-54 and zinc-65 had been added. The algae culture remained undisturbed at room temperature for two weeks

after which six 250 ml aliquots were removed and centrifuged at 5,000 rpm. The separated algae were resuspended in small quantities of lake water and spread evenly over a 2.5 cm layer of sediment in the bottoms of six aquaria. The mud was allowed to dry partially in order to fix the algae at the surface. Lake water, 500 ml, was carefully added to each aquarium. Approximately 200 amphipods were put into two of these aquaria and the cultures were maintained for 12 hours at 10° C. Two aquaria containing sediment, labeled algae and lake water were stored for 5 days at 10° C. After this period 200 similar sized amphipods were placed in each of these aquaria. These cultures were maintained at 10° C for 12 hours. After a storage period of 10 days the remaining two aquaria received 200 amphipods. These cultures were also maintained at 10° C for 12 hours. The accumulation multiples of all three radionuclides were computed, and used as indices of the efficiency of uptake by P. affinis of radionuclides associated with the algae.

5. Size of P. affinis (Experiment-12)

An aquarium containing lake water, sediment, and 10^{-3} $\frac{\mu Ci}{ml}$ each of strontium-85, manganese-54 and zinc-65, and approximately 370 P. affinis was maintained at 10° C for 72 hours. The P. affinis were then removed, spread out on absorbent paper, and separated into two groups according to apparent size. Microscopic examination of representative samples of each group showed that they had been divided into groups with a range of overall uncurled lengths of 4.0-6.5 mm and 8.0-10.5 mm exclusive of antennae. All amphipods were weighed and counted using the gamma spectrometer. Accumulated radionuclide concentrations, of amphipods were calculated.

CHAPTER V

EXPERIMENTAL RESULTS

A. Radionuclides Accumulated by *P. affinis* (Experiments 1 and 2)

The gamma spectra of the liquid wastes from the chemical reprocessing plant and the nuclear power plant are shown in Figure 7. The concentrations of radionuclides are indicated on these spectra. Exposure of *P. affinis* to dilute solutions of these wastes for 24 hours resulted in the accumulation of manganese-54 and zinc-65 by the amphipods. Accumulation multiples in *P. affinis* resulting from the 24 hours of exposure were 300 for manganese-54 and 410 for zinc-65.

Of the radionuclides used in Experiment-2 *P. affinis* accumulated detectable amounts of manganese-54, zinc-65 and strontium-90 (indicated by gross beta counts). Levels of other accumulated radionuclides were less than the corresponding background activities. *P. affinis* were exposed for 72 hours to concentrations of 124 pCi/ml of manganese-54, 7.0 pCi/ml of zinc-65 and 3 pCi/ml strontium-90. The accumulation multiples resulting from this exposure were 250 for manganese-54, 247 for zinc-65 and 41 for strontium-90.

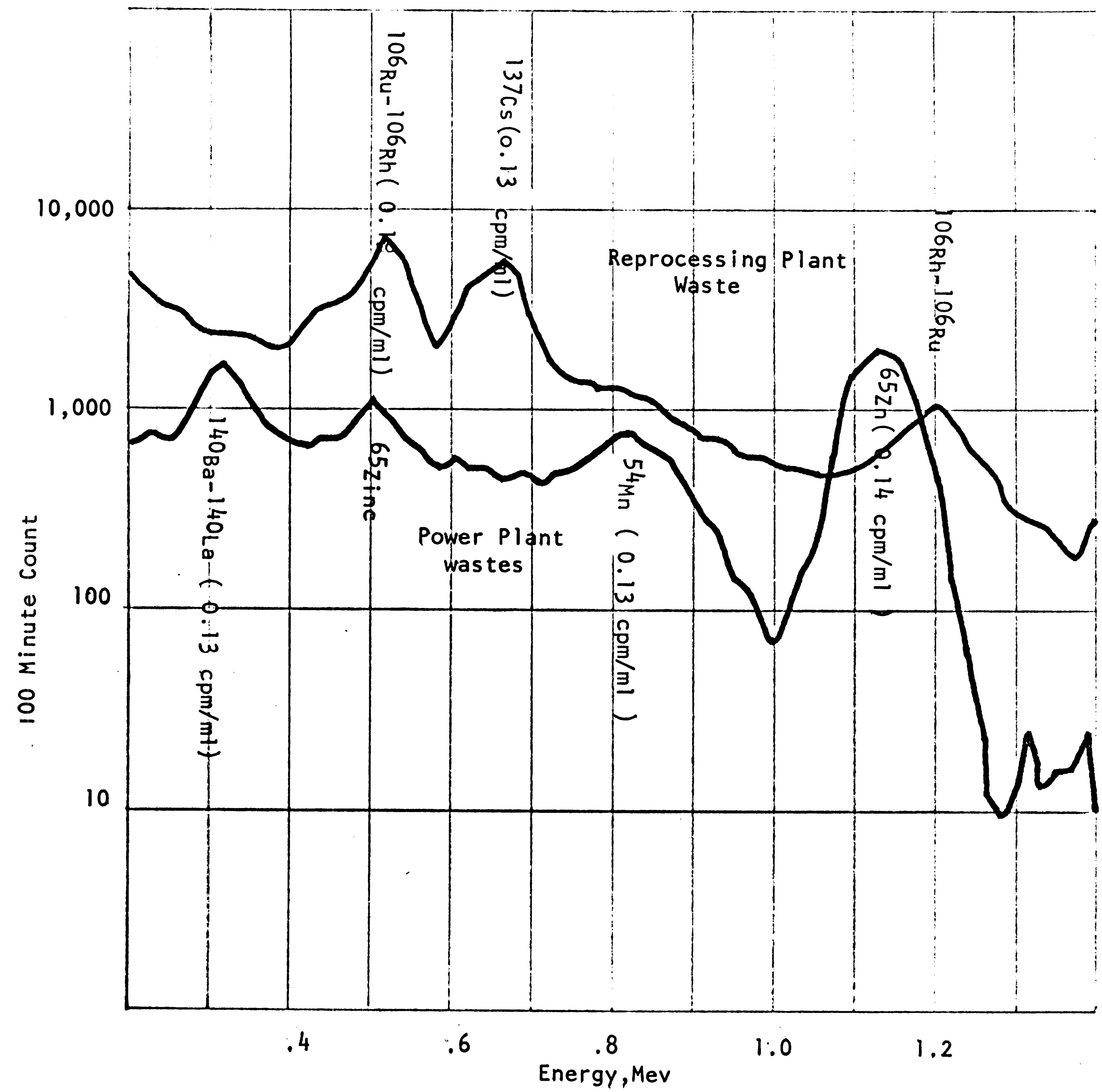

FIGURE 7. GAMMA-RAY SPECTRA FOR LIQUID WASTES FROM NUCLEAR FACILITIES. (3ml VOLUMES COUNTED ON A FLAT NaI(Tl) CRYSTAL)

B. Stable Element Concentrations in Lake Water, P. affinis and Fish Flesh

1. Stable element concentrations (Experiment-3)

The concentrations of strontium, manganese and zinc in Lake Michigan water, P. affinis and L. cephalus are listed in Table 4. The concentrations of manganese and strontium in L. cephalus were below the sensitivity of the absorption unit and the limiting concentrations were indicated.

TABLE 4

CONCENTRATION OF STABLE ELEMENTS IN PONTOPOREIA AFFINIS, L. CEPHALUS AND LAKE MICHIGAN WATER (EXPERIMENT-3)

Sample	Stable element concentrations ± Std. error Strontium	 Manganese	 Zinc
lake water*	110 ± 55.0 ppb	2.50 ± 0.90 ppb	10.0 ± 2.00 ppb
P. affinis	28.6 ± 9.54 ppm	13.9 ± 5.00 ppm	35.4 ± 10.3 ppm
L. cephalus (flesh)	< 0.37 ppm	< 0.19 ppm	5.46 ± 1.46 ppm

*See Appendix A for additional concentrations of elements in Lake Michigan water.

2. Concentration factors

The naturally occuring concentrations of manganese, strontium and zinc in young adult P. affinis and L. cephalus analyzed in Experiment-3 are considered equilibrium concentrations. Dividing these concentrations by the corresponding elemental concentrations in lake water yielded the

concentration factors. The concentration factors for strontium manganese and zinc in the flesh of L. cephalus were < 3.4, < 80 and 546 respectively. These results indicate the existence of some factor or factors which discriminates against the transfer of radionuclides up the food chain.

3. Projected maximum consumption of fish flesh

The maximum permissible consumption rates of the flesh of L. cephalus were determined for fish at equilibrium with water containing the limiting concentrations of strontium-90, manganese-54 and zinc-65 permitted in waters used by the general public. These calculations were made by substituting the concentration factors for L. cephalus into equation 4. These results indicated that the projected maximum permissible consumption of L. cephalus would be 4.0 g/day as determined by the equilibrium concentration of zinc-65.

Radioanalysis of whole fish (L. cephalus) taken in the vicinity of the Big Rock Nuclear Reactor revealed the presence of 1.88 pCi/g of zinc-65 and 1.07 pCi/g of cesium-137 (see Figure 8). Maximum permissible consumption of these fish was 41 kg/day as determined by substituting into equation 3.

C. Accumulation and Elimination of Strontium-85 Manganese-54 and Zinc-65 by Pontoporeia affinis

1. Results of accumulation experiments (Experiment-4)

The results of repeatedly exposing live P. affinis to levels of strontium-85, manganese-54 and zinc-65 equal to the corresponding MPC are shown graphically in Figure 9. It is apparent that equilibrium levels of

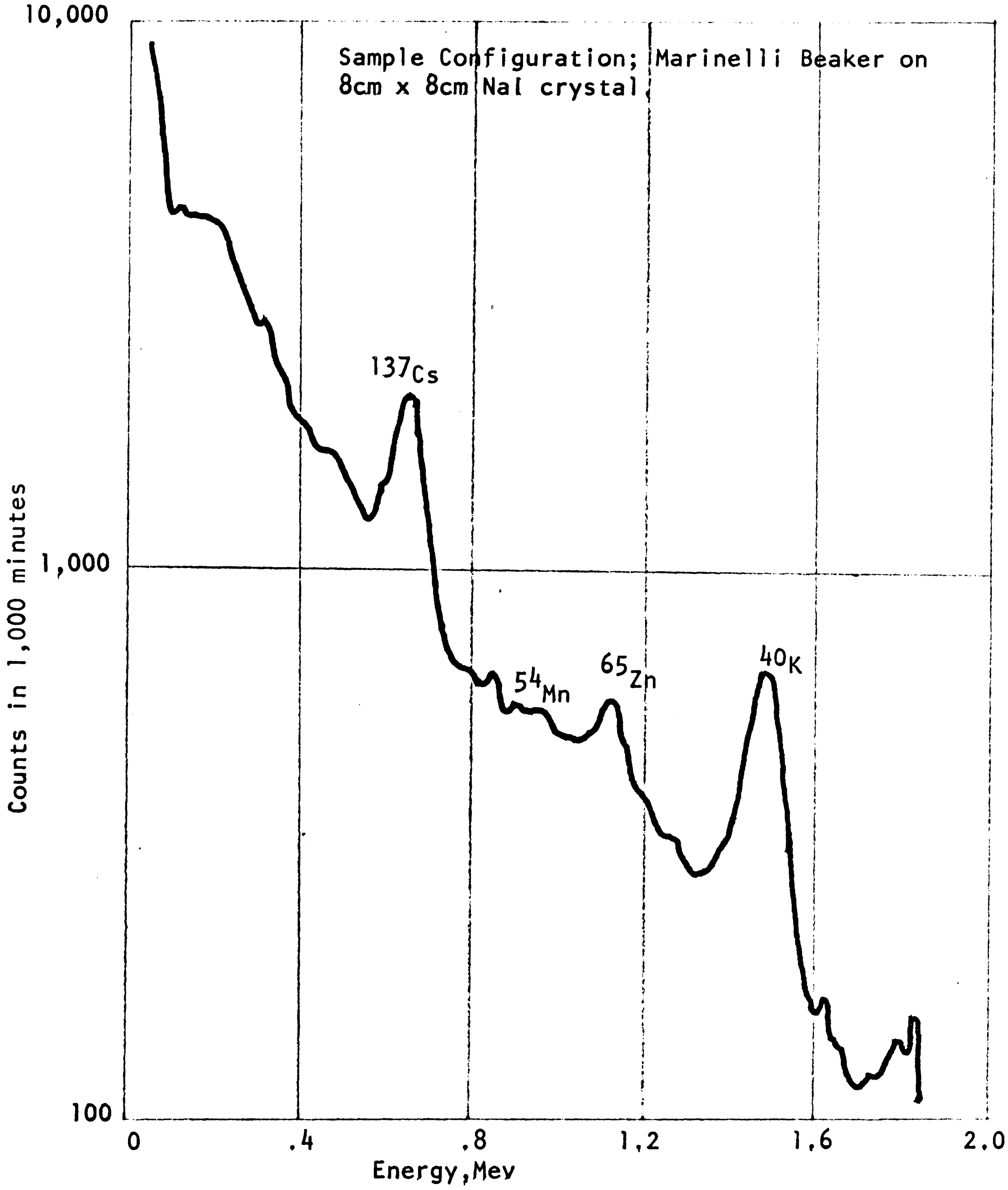

FIGURE 8. GAMMA-RAY SPECTRUM FOR 450g(wet wt.) OF CHUB (L. CEPHALUS) FLESH.

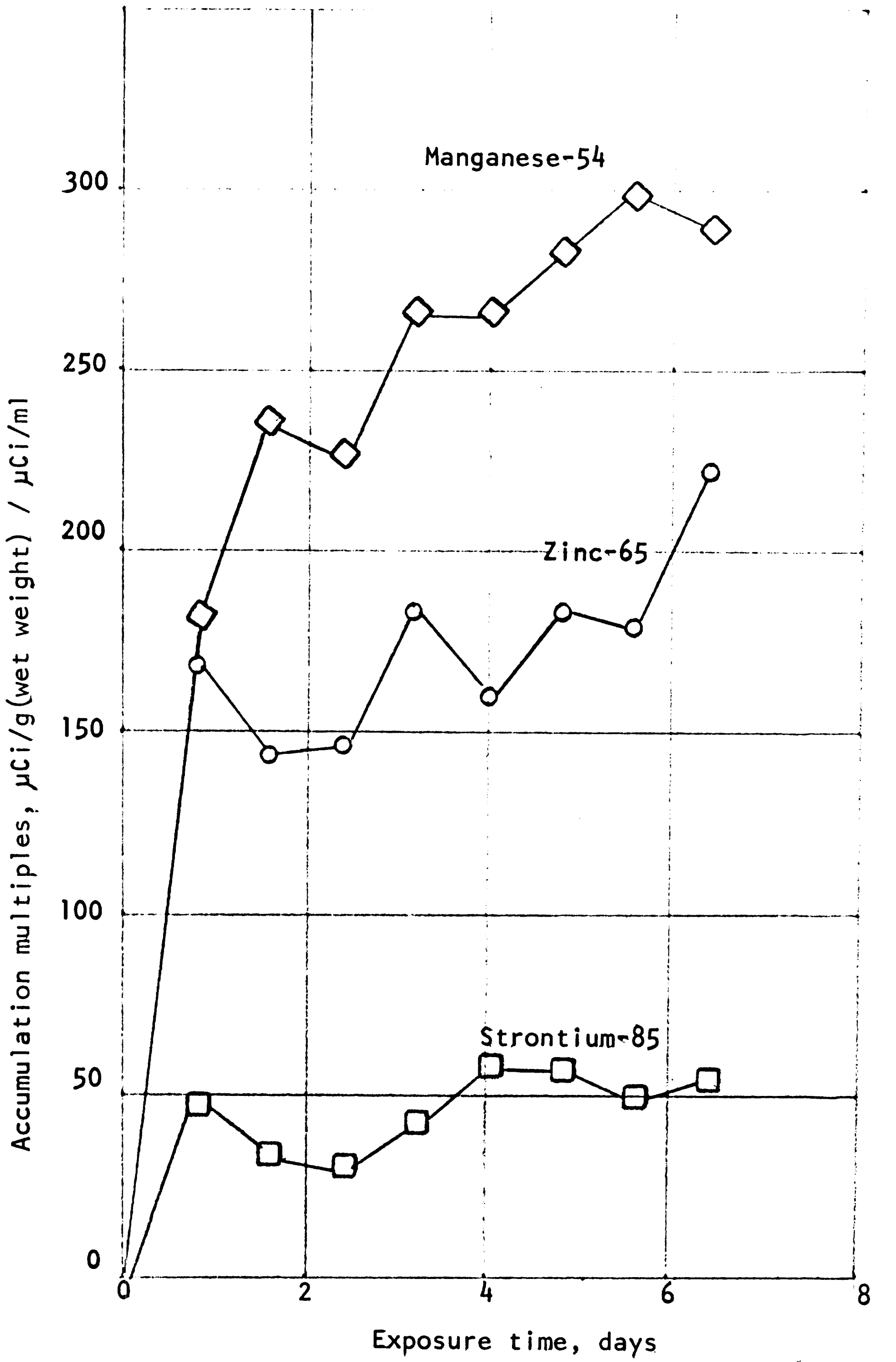

FIGURE 9. ACCUMULATION OF RADIONUCLIDES BY PONTOPOREIA AFFINIS EXPOSED TO THE REPEATED RELEASE OF 10^{-4} µCi/ml OF STRONTIUM-85, MANGANESE-54, AND ZINC-65. (Experiment #4)

of radionuclides corresponding to naturally occuring concentration factors are not obtained by P. affinis in this experiment. An average of 53 animals from each aquarium had died by the eighth day of the experiments and they were terminated. The shape of the accumulation curves indicated that the levels of accumulated radionuclides approached apparent equilibrium with respect to the laboratory environment. The maximum values of accumulation multiples achieved by P. affinis in these experiments were 60 for strontium-85, 300 for manganese-54 and 225 for zinc-65.

2. Results of elimination tests (Experiment-5)

Semi-logarithmic graphs depicting the percentages of radioactivity remaining in P. affinis versus the time the amphipods spent in clean sediment and water are shown in Figure 10. Similar curves for radioactive P. affinis in aquaria in which only the water was exchanged daily are also shown in Figure 10. The biological half-life, T_B, of each radionuclide accumulated by P. affinis was determined from the slope of the straight portion of the elimination curve for the amphipods in clean water and sediment. The lengths of time taken by the experimental animals to eliminate 50% of the initial body burdens of strontium-85, manganese-54 and zinc-65 were determined by direct inspection or extrapolation of the elimination curves in Figure 10. These periods referred to as T_{50} values, are shown adjacent the appropriate curves in Figure 10.

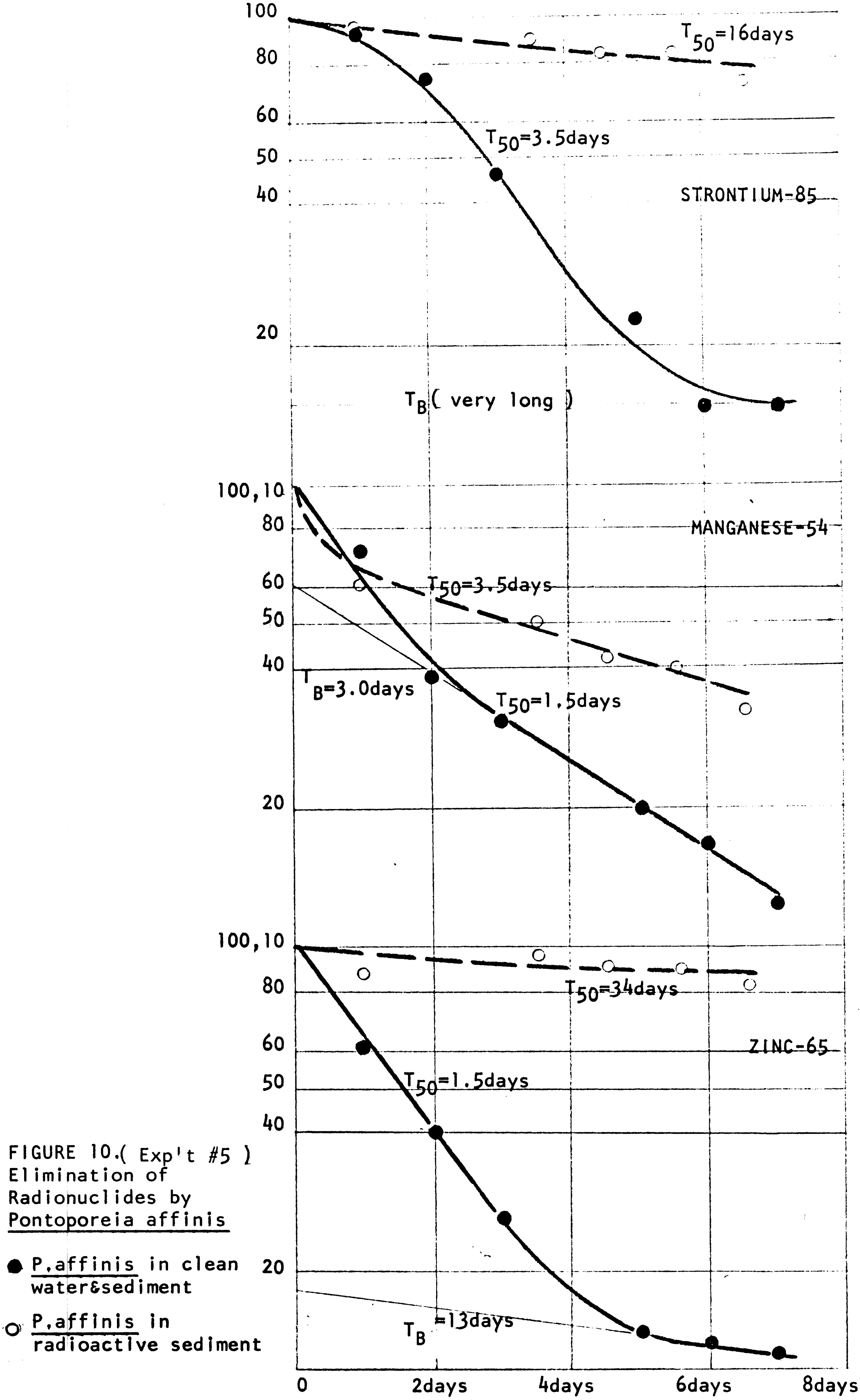

FIGURE 10.(Exp't #5)
Elimination of
Radionuclides by
Pontoporeia affinis

D. Distribution of Radionuclides Accumulated by P. affinis

1. Removable and non-removable radioactivity (Experiment-6)

The average fractions of strontium-85 and zinc-65 removed from radioactive P. affinis by preservative (formalin) solutions were 80% of the initial amphipod body burdens. An average of 43% of the accumulated manganese-54 was removed from experimental animals stored in formalin solutions. The standard deviations associated with all estimates of the quantities of removable and non-removable radionuclides were less than 7%.

2. Radionuclides in the exoskeleton of P. affinis (Experiment-7)

Microscopic examination of the chemically excised exoskeletons (Figure 11) of P. affinis revealed some undissolved soft tissue inside some shells. These were manually removed from the sample. The remaining exoskeletons were separated and analyzed for gamma radioactivity. Measurable quantities of zinc-65 and manganese-54 were not detected in any group or in the combined group of shells. A single measurement which indicated that 12% of the accumulated strontium-85 was in the exoskeleton could not be duplicated.

E. Influence of certain environmental factors on the Accumulation of Radionuclides by Pontoporeia affinis

1. Temperature effects (Experiment-8)

The temperature response curves in Figure 12 show the effects of temperature on the accumulation of strontium, manganese and zinc by

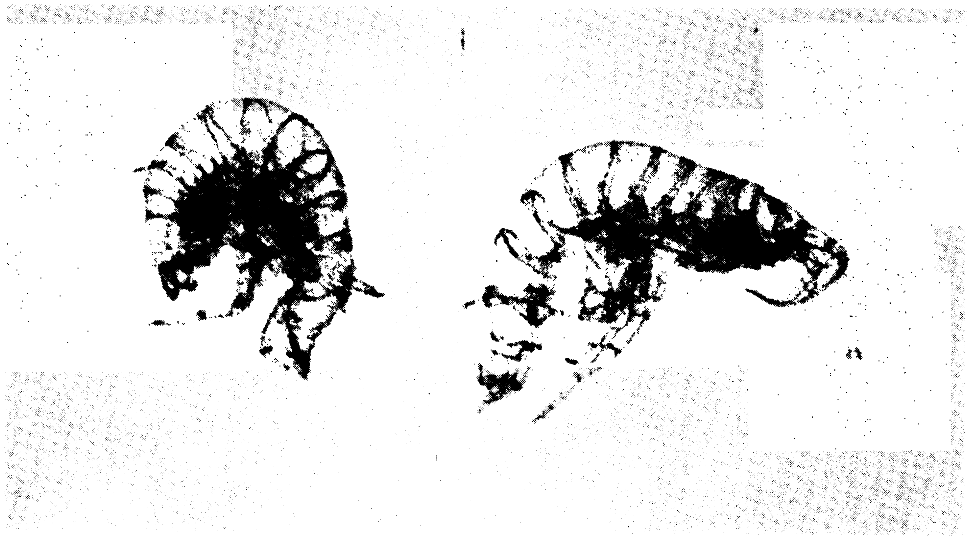

FIGURE 11. CHEMICALLY EXCISED EXOSKELETONS OF PONTOPOREIA AFFINIS.

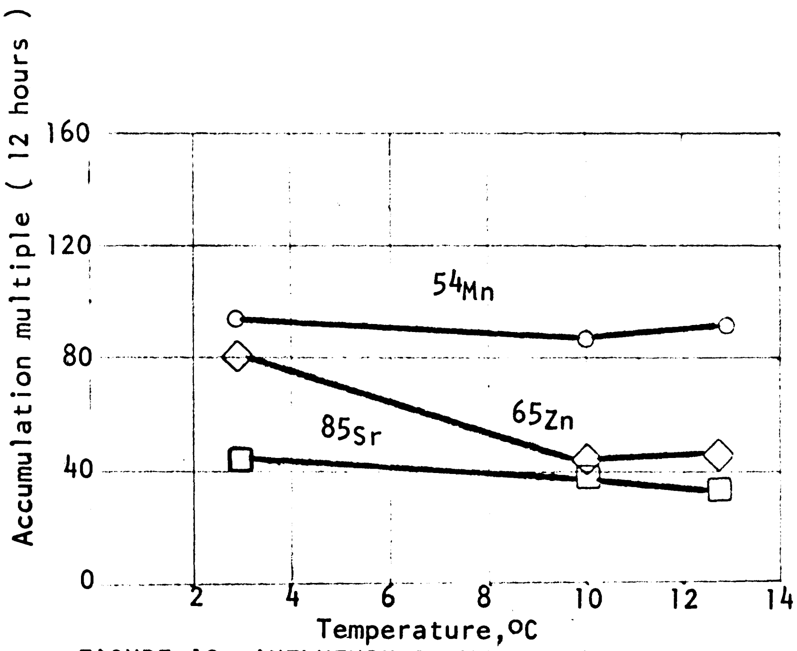

FIGURE 12. INFLUENCE OF TEMPERATURE ON RADIONUCLIDE ACCUMULATION BY PONTOPOREIA AFFINIS AT pH = 7.8. (Experiment #8)

P. affinis. Over the range of temperatures from 3° C to 13° C significant variations in the accumulation of manganese-54 and strontium-85 were not observed in P. affinis. The reduction in accumulation was more pronounced in the case of zinc-65, but also not statistically significant. Many of the amphipods maintained at 20° C did not survive and others appeared sluggish. The radionuclide accumulation was not determined for these animals.

2. pH effects (Experiment-9)

The curves in Figure 13 show that the accumulation efficiencies of strontium, manganese and zinc in P. affinis were increased when the pH of aquaria waters was increased from 5.5 to 7.8. The accumulation of manganese increased further when the pH was raised to 9.3 while accumulation efficiencies for strontium and zinc realized at pH 7.8 were reduced by 93%, and 60% respectively.

3. Results of radionuclide routing experiment (Experiment-10)

The final distribution of radionuclides used in the routing experiments is shown in Table 5. The quantities of radionuclides in P. affinis reported in this table were amounts taken up in 12 hours less the gut content. The percentages of radioactivity removed by P. affinis indicate the efficiency with which the amphipods accumulated radionuclides originally located in the different phases of their laboratory environments, i.e. water, sediment, and sterilized sediment.

Strontium-85 is accumulated most effectively by way of the water route. The introduction of bacterially active sediment into the laboratory environments reduced the strontium accumulation efficiency

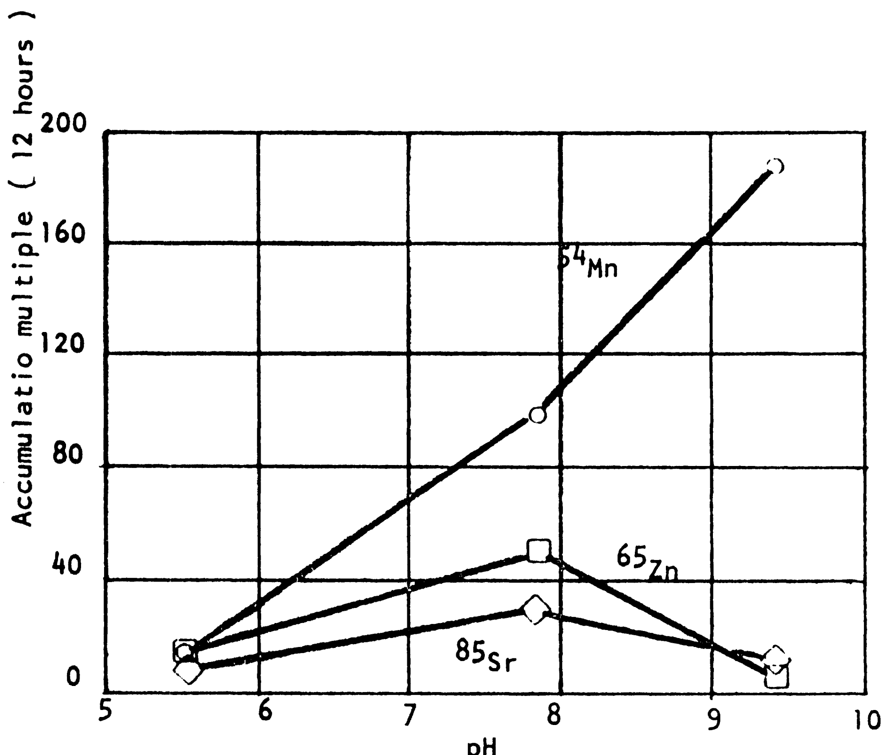

FIGURE 13. VARIATION OF RADIONUCLIDE ACCUMULATION BY PONTOPOREIA AFFINIS WITH pH.AT TEMPERATURE = 10°C. (Experiment #9)

demonstrated in water by Pontoporeia by 30%. The accumulation efficiency of P. affinis for strontium-85 associated with radioactive sediment was increased by 29% when the sediment was sterilized.

Laboratory environments containing only radioactive water constituted the most efficient route for the accumulation of manganese-54 by P. affinis. This efficiency was reduced by 27% with the introduction of bacterially-active sediment. The efficiency with which P. affinis accumulated manganese-54 from sediment was reduced by 47% by sterilization of the sediment.

Zinc-65 was most effectively accumulated by P. affinis from laboratory environments containing only radioactive water. The introduction of bacterially-active sediment reduced this efficiency 33%. P. affinis accumulation efficiency for zinc-65 in the sediment was decreased by 63% by sterilizing the sediment.

The 12-hour accumulation multiples for P. affinis and radionuclides involved in all routing tests are shown in Table 6. Accumulation multiples for strontium-85, manganese-54 and zinc-65 located in the bacterially active sediment are greater than those realized via other routes.

4. The influence of settled organic matter on radionuclide accumulation by P. affinis (Experiment-11)

P. affinis exposed to radioactive algae associated with the sediment in test aquaria did not accumulate significant quantities of manganese-54 and zinc-65 until some decomposition of the algae had taken place. The quantity of strontium-85 accumulated by P. affinis in this experiment did not vary significantly when the algae substrate was aged. Figure 14 shows the results of all tests in this experiment. The

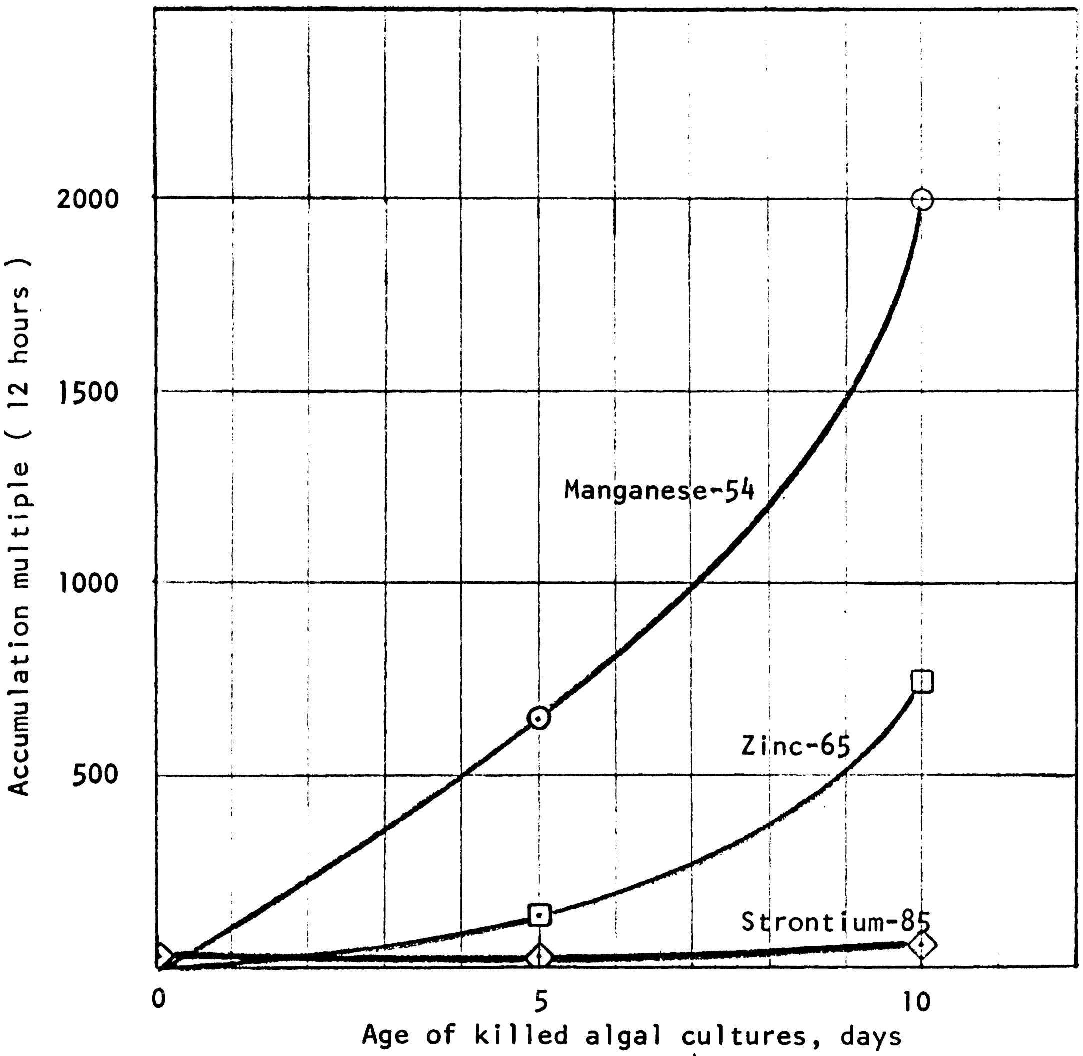

FIGURE 14. ACCUMULATION OF RADIONUCLIDES BY PONTOPOREIA AFFINIS EXPOSED TO RADIOACTIVE ORGANIC MATTER. (Experiment #11)

TABLE 5

FINAL DISTRIBUTION OF RADIONUCLIDES IN ROUTING TESTS (EXPERIMENT 10)

Route*	TOTAL µCi	FINAL DISTRIBUTION OF RADIOACTIVITY					
		WATER		SEDIMENT**		PONTOPOREIA	
		µCi	%	µCi	%	µCi	%
	STRONTIUM-85						
WS	28.4	20.9	73.5	7.23	25.6	0.27	0.95
WO	25.0	24.6	98.6	NO SEDIMENT		0.34	1.36
NS	3.60	1.25	34.7	2.32	64.5	0.03	0.83
HS	2.80	1.55	55.6	0.92	43.4	0.03	1.07
	MANGANESE-54						
WS	28.0	12.0	42.9	15.5	55.3	0.50	1.80
WO	20.5	20.0	97.5	NO SEDIMENT		0.50	2.50
NS	7.80	0.31	3.98	7.43	95.3	0.06	0.77
HS	2.40	0.58	24.2	1.81	75.4	0.01	0.41
	ZINC-65						
WS	31.0	1.87	60.3	12.0	38.8	0.25	0.81
WO	24.8	24.5	98.8	NO SEDIMENT		0.30	1.31
NS	6.00	1.14	19.0	4.79	79.8	0.07	1.17
HS	1.80	1.66	92.2	0.13	7.23	.008	0.44

TABLE 5--Continued

*Route refers to the original location of radioactivity in:
WS - water and sediment
WO - water only
NS - natural sediment
HS - heat treated sediment

**Sediment content determined by subtraction.

TABLE 6

ACCUMULATION MULTIPLES DEVELOPED IN PONTOPOREIA USED IN ROUTE TESTS

Route*	Isotope	Concentration in *P. affinis*** µCi/g	Concentration in Water** µCi/g	Accumulation Multiple
WS	^{85}Sr	0.27	7.0×10^{-2}	4.86
	^{54}Mn	0.50	4.0×10^{-2}	12.5
	^{65}Zn	0.25	6.2×10^{-2}	4.04
WO	^{85}Sr	.34	8.2×10^{-2}	4.15
	^{54}Mn	.41	6.6×10^{-2}	7.57
	^{65}Zn	.38	8.2×10^{-2}	3.53
NS	^{85}Sr	0.03	0.4×10^{-2}	7.5
	^{54}Mn	0.06	0.1×10^{-2}	60
	^{65}Zn	0.07	0.4×10^{-2}	17.5
HS	^{85}Sr	0.03	0.5×10^{-2}	6.0
	^{54}Mn	0.01	0.2×10^{-2}	5.0
	^{65}Zn	.008	0.5×10^{-2}	1.6

*See footnote, Table 5.

**25 Pontoporeia (100 mg wet wt.) 300 ml lake water in each aquarium (Experiment-10).

accumulation multiples realized by *P. affinis* during 12 hours exposure at 10° C to 10-day old radioactive algae were 54, 2,000 and 725 for strontium-85, manganese-54 and zinc-65 respectively.

5. The influence of amphipod size on radionuclide accumulation (Experiment-12)

The experimental results given in Table 7 indicate that the average concentrations of all radionuclides used were greater in small (3.5 mg wet weight) *P. affinis* than in larger (6.0 mg wet weight) amphipods. Consideration of statistical variations indicate, however, that these differences are not highly significant.

TABLE 7

ACCUMULATED RADIONUCLIDES IN *P. AFFINIS* AS RELATED TO SIZE (EXPERIMENT-12)

Average Amphipod Wet Weight, mg	Average Accumulated radioactivity, cpm/mg ± 0.95 Error		
	Strontium-85	Manganese-54	Zinc-65
3.5	42 ± 21	53 ± 26	71 ± 29
6.0	30 ± 17	21 ± 8	48 ± 19

CHAPTER VI

DISCUSSION OF RESULTS

The facts presented in Chapter III of this dissertation concern the ecology of P. affinis in Lake Michigan. They establish that P. affinis is the most abundant and widely distributed benthic organism in Lake Michigan, and that the amphipod lives longer than 1 year. Alley (1968) reports that although adult P. affinis are strong swimmers there was no apparent migration between sampling areas approximately 4 m apart. The foregoing facts indicate that P. affinis meets two of the criteria for a suitable monitor organism stated in the introduction. There is the additional advantage that routine sampling and analyses of P. affinis could reflect seasonal variations in environmental radioactivity. It is also possible and may in certain instances be desirable to transplant P. affinis from their natural habitat to other locations in Lake Michigan for investigative purposes.

The radionuclides accumulated by P. affinis have been observed at significant levels in other marine and fresh-water benthic organisms. More often these radionuclides have been part of enzymatic systems, trapped between muscle fibers or within the crystalline structure of calcareous tissue. The naturally occurring concentration factors determined in the present study for strontium, manganese and zinc in P. affinis were of the same order of magnitude as corresponding concentration factors in other fresh-water invertebrates. Some of these values were compared in the following table:

TABLE 8

CONCENTRATION FACTORS IN FRESH-WATER INVERTEBRATES

Organism	Strontium	Manganese	Zinc
P. affinis (amphipod)[a]	260	5,840	3,540
Hydropsychidae (caddis fly larvae)[b]	--	4,700	6,000
Daphnia magna (cladoceran)[c]	150	--	--
Axiopas (amphipod)[d]	--	--	2,000
Lampsilis radiata (clam)[e]	--	2,400	4,100

- Not determined.

[a]Present study (Table 4)

[b]Watson (1967)

[c]Marshall et al. (1964).

[d]Phelps et al. (1969).

[e]Harvey (1969).

Sampling procedures for benthic organisms used by scientists of the University of Michigan's Great Lakes Research Division involved approximately $0.2m^2$ of bottom surface. The standing crop of P. affinis within the sampling area equaled 2.6 g (wet weight). The second of the criteria for a suitable monitor organism stated in the introduction to this dissertation requires that the 2.6 g of P. affinis must contain at least that quantity of a radionuclide in 3.5 liters of lake water. Therefore, the minimum concentration factor needed to monitor a radionuclide using P. affinis is 1,350. The concentration factors for

manganese and zinc determined in this study were above the minimum level, but the concentration factor for strontium in P. affinis was well below the required level.

The loss of each accumulated radionuclide by P. affinis was characterized by two modes of elimination. The portions of each accumulated radionuclide eliminated at the faster rates (Experiment 5, p. 38) were approximately equal to the removable fractions of accumulated radioactivity measured in Experiment-6. Based on the assumption of the first order reaction kinetics (Polikarpov 1967 and Marshall et al. 1964) the times required for P. affinis to reach a steady-state condition with respect to the laboratory environment was estimated using T_{50} values from Figure 10. Manganese-54 and Zinc-65 would reach 99% of their equilibrium values in 10 days, and 23 days would be required for the equilibration of strontium-85 in P. affinis. These attainment times satisfy the requirement for equilibration in 1 month. The recycling of radionuclides to P. affinis from contaminated sediment (Figure 10, dashed curves) demonstrates the influence of sediment on the measured amounts of radionuclides in P. affinis. The extended elimination times caused by this recrycling improve the "memory" of P. affinis for contaminating events. The maximum accumulation multiples for strontium-85, manganese-54 and zinc-65 in P. affinis observed in Experiment-4 were significantly lower than the corresponding naturally occuring concentration factors. This was probably due to absence in the laboratory environments of the biological mechanisms responsible for the sustained transport of radioactive organic material from the overlying water column to natural benthic environments.

The stable element measurements of Experiment-3 indicated that there were natural factors which limited the transfer of strontium,

manganese and zinc from P. affinis to L. cephalus, i.e., up the food chain. Possible explanations of this situation would be the movement of L. cephalus and/or the presence of zinc in tissues of P. affinis which are indigestable to L. cephalus. Despite the inefficient nature of the transfer of zinc-65 from P. affinis to L. cephalus this transfer may be the critical factor in the food chain involving man. As the results of Experiment-3 indicated, the maximum permissible continuous consumption of the flesh of L. cephalus (chubs) at equilibrium with MPC levels of zinc-65 in lake water would be 4.0 g/day, i.e., approximately 4 lbs per year. It is highly probable that people who eat fish regularly (at least once per week) could consume 4 lbs of zinc-65 - contaminated fish flesh in 1 month or less. Since fish are not the only source of ingested radioactivity the consideration of zinc-65 contamination in L. cephalus is important.

The results of the radionuclide routing tests (Experiment-10) and algae feeding (Experiment-11) revealed that environmental water was the source of strontium-85 accumulated by P. affinis. Routing tests also showed that the destruction of some biologic aspect of the sediment reduced the accumulation of manganese-54 and zinc-65 by P. affinis. The fact that the accumulation of manganese-54 and zinc-65 by P. affinis in experiment-11 increased geometrically with linear increases in the age of the algae substrate suggested that the amphipod's accumulation of these isotopes might have been directly related to the growth of bacterial populations since benthic amphipods have been observed by Wisen (1956) and Hargrave (1970) to consume bacteria found on the surfaces of particulate organic material.

The variations of temperature (experiment-8) and amphipod size (experiment-12) observed in this study did not significantly alter the levels of accumulation of strontium-85, manganese-54 and zinc-65 by P. affinis. The reduction of strontium-85 and zinc-65 accumulation at pH 9.3 was probably due to the formation of $CaCO_3$ and the co-precipitation of ionic zinc and strontium. The co-precipitation of ionic zinc on $CaCO_3$ was observed by Bachmann (1963).

Radiation damage to experimental animals was considered negligible because the dose to P. affinis was 1.30×10^{-3} RADS per hour (see calculations, Appendix C). This dose rate was well below the 0.32 RADS per hour observed by Marshall (1962) to cause the loss of filter-feeding capacity in the fresh-water crustacean Daphnia pulex.

CHAPTER VII

CONCLUSIONS

The foregoing interpretation of experimental results observed during this investigation led to the following conclusions relative to the questions stated in the introduction to this dissertation:

1. P. affinis is a suitable monitor organism for manganese-54 and zinc-65;

2. P. affinis is not a suitable monitor organism for strontium-85 or either of its longer-lived isotopes, strontium-89 or strontium 90; and

3. The quantity of radionuclides in the flesh of L. cephalus (chubs) did not constitute a radiological health hazard to man. However, continuous consumption of more than 4 g per day of L. cephalus in equilibrium with water containing 10^{-4} μCi/ml of zinc-65 would constitute a radiological health hazard.

The observations made during the present investigation and the experimental results presented here indicate that research into the following areas would yield answers to other questions of radioecological importance:

1. efficiency of radionuclide transfer from radioactive P. affinis to edible fish flesh

2. radionuclide accumulation by P. affinis from labeled aquatic bacteria

3. suitability of other sessile aquatic organisms, i.e., clams, benthic worms, periphyton, etc., for localized use as monitor organisms

4. effects of long exposure to low concentrations of radioactive substances in the benthic environment on the population dynamics of P. affinis.

REFERENCES

1. Alley, W. P., Ecology of the Burrowing Amphipod Pontoporeia affinis in Lake Michigan, A Ph.D. Thesis written at the University of Michigan. 1968.

2. Armstrong, N. E. and Gloyna, E. F., "Mathematic Models for The Dispersion of Radionuclides in Aquatic Systems" Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 329-335.

3. Ayers, J. C., Big Rock Nuclear Power Plant Hydrological Survey, The University of Michigan, Great Lakes Research Division, Spec. Rep. No. 9 (1961).

4. Ayers, J. C., "Great Lakes Water, Their Circulation and Physical and Chemical Characteristics," Great Lakes Basin Pub. No. 71 of the American Association for the Advancement of Science (1962) 71-89.

5. Bachmann, R. W., "Zinc-65 in Studies of the Fresh-water Zinc Cycle," Radioecology, V. Schultz and A. W. Klement, Jr., (Eds.) Reinhold, N. Y. (1963) 485-496.

6. Bennett, R. R., "Planning for Power....A Look at Tomorrows Station Sizes," I. E. E. Spectrum, (Sept. 1968) 67-72.

7. Brey, W. S., Principles of Physical Chemistry, edited by R. S. Halford, A. C. C. Inc., N. Y. (1958).

8. Comar, C. L. and Lengemann, F. W., "General Principles of the Distribution and Movement of Artificial Fallout Through the Biosphere to Man," Radioecological Concentration Processes, edited by Aberg, B., and Hungate, F. P., Pergamon Press (1967).

9. Cross, F. A., Dean, J. D., and Osterberg, C. L., "The Effect of Temperature, Sediment, and Feeding on the Behavior of Four Radionuclides in a Marine Benthic Amphipod," Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 450-462.

10. Duke, T., Willis, J., Price, T. and Fischer, K., "Influence of Environmental Factors on the Concentrations of Zinc-65 by an Experimental Community," Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 355-363.

11. Eggleton, F. E., A Limnological Study of the Profundal Bottom Fauna of Certain Fresh-water Lakes, Ecological Monographs Vol. 1-3 (1931) 233-331.

12. Ferguson, R. G., "Bathymetric Distribution of American Smelt" Proc. 8th Conf. Great Lakes Research, University of Michigan Great Lakes Research Division Pub. No. 13 (1965) 132-137.

13. Fowler, S. W., Small, L. F., and Dean, J. M., "Metabolism of Zinc-65 in Euphausiids," Symposium on Radioecology, edited by Nelsen, D. J. and Evans, F. C., U. S. Atomic Energy Commission Division of Biology and Medicine, TID-4500 (1969) 399-412.

14. Gustafson, P. F., "Comments on Radionuclides in Aquatic Ecosystems," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press (1967) 853-859.

15. Hargrave, B. T., "The Effect of a Deposit-Feeding Amphipod on the Metabolism of Benthic Microflora, Limnology and Oceanography, Vol. 15 No. 1, 21-31 (Jan. 1970).

16. Harvey, R. S., "Uptake and Loss of Radionuclides by the Freshwater Clam Lampsilis Radiata (Gmel.)," Health Physics Pergamon Press (1969) Vol. 17, 149-154.

17. Johannes, R. E., "Release of Phosphorus by a Benthic Marine Amphipod," Limnology and Oceanography, Vol. 9 No. 2 (April 1964) 235-241.

18. Lellak, J., "The Food Supply as a Factor Regulating the Population Dynamics of Bottom Animals," Journal of the Intrnl. Assn. of Theoretical and Applied Limnology (1965) 120-136.

19. Marshall, J. S., "The Effects of Continuous Gamma Radiation on the Intrinsic Rate of Natural Increase of Daphnia Pulex," Ecology, Vol. 43 No. 4 (1962) 598-607.

20. Marshall, J. S., Beeton, A. M. and Chandler, D. C., "Role of Zooplankton in the Freshwater Strontium Cycle and Influence of Dissolved Salts," Verh. Internat. Verein. Limnol. XV (1964) 665-672.

21. Mason, W. H. and Odum, E. P., "The Effect of Coprophagy on Retention and Bioelimination of Radionuclides by Detritus-Feeding Animals," Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 721-725.

22. Marzolf, G. R., Substrate Relations of The Burrowing Amphipod Pontoporeia affinis (Lindstrom) A Ph.D. Thesis in the University of Michigan (1963) 1-92.

23. Merlini, M., "The Freshwater Clam as a Biological Indicator of Radiomanganese," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press (1967) 977-983.

24. Pelletier, C. A., "The Sensitivity of Environmental Surveys to Atmospheric Releases From Nuclear Facilities," A Ph.D. Thesis in the University of Michigan (1966) 1-154.

25. Pennak, R. W., Fresh-water Invertebrates of the United States, Ronald Press Co. (1953) 435-446.

26. Phelps, D. K. "Partioning of the Stable Elements, Fe, Zn, Sc, and Sm within a Benthic Community, Anasco Bay, Puerto Rico," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press (1967) 721-735.

27. Phelps, D. K., Santiago, R. J., Luciano, D. and Irizarry, N., "Trace Element Composition of Inshore and Offshore Benthic Populations," Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 509-527.

28. Polikarpov, G. G., Zaitsev, V. P., Barinov, G. V. and Parchevsky, V. P., "General Features of Concentration Processes of Radioactive Substances by Hydrobionts in Different Seas of the World," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press _1967) 771-791.

29. Polikarpov, G. G., "Regularities of Uptake and Accumulation of Radionuclides in Aquatic Organisms," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press (1967) 819-827.

30. Powers, C. F., and Alley, W. P., "Some Preliminary Observations on the Depth Distribution of Macrobenthos of Lake Michigan," University of Michigan, Great Lakes Research Division Special Report No. 30 (1967) 112-125.

31. Powers, C. F., and Robertson, A., "Some Quantitative Aspects of The Macrobenthos of Lake Michigan," Pub. No. 13, Great Lakes Research Division, The University of Michigan (1965) 153-159.

32. Preston, A., "The Concentration of Zinc-65 in the Flesh of Oysters Related to the Discharge of Cooling Pond Effluent from the C. E. G. B. Nuclear Power Station at Bradwell-on-Sea, Essex," Radioecological Concentration Processes, edited by Aberg, B. and Hungate, F. P., Pergamon Press (1967) 995-1005.

33. Reed, J. R. and Nelson, D. J., "Radiostrontium Uptake in Blood and Flesh in Bluegills (Lepomis Machirus)," Symposium on Radioecology, edited by Nelson, D. J. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 227-239.

34. Renfro, William C., Phelps, R. V. and Guthrie, T. F., "Influence of Preservation and Storage on Radiozinc and Total Zinc Concentration in Fish," Limnology and Oceanography, Vol. 14 No. 1 (1969).

35. Risely, C., "Radioactivity in Lake Michigan and its Tributaries," Proc. 8th Conf. Great Lakes Research, The University of Michigan, Great Lakes Res. Div. Special Rep. No. 13 (1965) 122-131.

36. Robertson, A. and Alley, W. P., "A Comparative Study of Lake Michigan Macrobenthos," Limnology and Oceanography, Vol. 11 No. 4 (1966) 576-583.

37. Rossman, R. and Callender, E., "Geochemistry of Lake Michigan Manganese Nodules," Proc. 12th Conf. Great Lakes Res. (1969) 306-316.

38. Ruttner, F., Fundamentals of Limnology, Univ. of Toronto Press (1953).

39. Schmitt, W. L., Crustacea, University of Michigan Press (1965) 1-204.

40. Segerstråle, S. G., "Synopsis of Data on the Crustaceans Gammarus locusta, Gammarus oceanicus, Pontoporeia affinis, and Corophim volutater (Amphipoda Gammaridea)," Soc. Sci. Fenn. Comm. Biol. Vol. 20 (1959) 1-23.

41. Segerstråle, S. G., "The Amphipods of the Coast of Finnland....Some Facts and Problems, Soc. Sci. Fenn. Comm. Biol. Vol. 10 (1950) 1-28.

42. Small, L. F., "Experimental Studies on the Transfer of Zinc-65 in High Concentrations by Euphausiids," Journal of Experimental Biology and Ecology, Vol. 3, 106-123 (1969).

43. Wangersky, P., "Manganese In Ecology," Radioecology, V. Schultz and A. W. Klement, Jr. (Eds), Reinhold, New York (1963) 113-120.

44. Watson, D. G., Cushing, C. E., Coutant, C. C., and Templeton, W. L., "Effect of Hanford Reactor Shutdown on Columbia River Biota," Symposium on Radioecology, edited by Nelson, D. F. and Evans, F. C., U. S. Atomic Energy Commission, Division of Biology and Medicine, TID-4500 (1969) 291-306.

45. Wells, L., "Seasonal Abundance and Vertical Movement of Planktonic Crustacea in Lake Michigan," Fisheries Bulletin, U. S. Fish and Wildlife Service, Vol. 60 (1960) 343-369.

46. Wigglesworth, V. B., Insect Physiology, J. Wiley and Sons (1956) 1-78.

47. Wisen, W., "Factors Influencing the Choice of Substratum in Cumella vulgarie," Limnology and Oceanography, Vol. 1 (1956) 274-285.

APPENDIX A

CHEMICAL ANALYSES OF LAKE MICHIGAN WATER

TABLE 9

ANALYSES OF LAKE MICHIGAN WATER (PPM)

	1907*	1969**
Calcium	26	36
Magnesium	8.2	12
Sodium-Potassium	4.7	5.4
Chloride	2.7	11
Sulfate	7.2	18.5

*See Ayers (1962).

**Callendar, U. of M. Great Lakes Res. Div.

TABLE 10

TRACE ELEMENT CONCENTRATIONS IN LAKE MICHIGAN (PPB)

Element	(1)	(2)
Manganese	2.0	2.5
Iron		15
Copper	5	2.9
Nickel	5	6.0
Zinc	10	10
Strontium		110
Cobalt		3.1

(1) Reported by Rossman (1969).

(2) Present study.

APPENDIX B

CALCULATION OF INTERFERENCE CORRECTIONS FOR COMPOUND GAMMA SPECTRA

For strontium-85, manganese-54 and zinc-65 we can define the following interference factors:

$$Fms = \frac{Sm}{Mm} = \frac{\text{contribution of strontium-85 source to Mn portion}}{\text{contribution of manganese-54 source to manganese-54 portion of the gamma spectrum.}}$$

Other factors are:

$Fsm = Ms/Ss$, $Fzm = Mz/Zz$, $Fmz = Zm/Mm$

$Fsz = Zs/Ss$, $Fzs = Sz/Zz$

Selected photo peak areas from 100 channel (0.02 Mev/Ch.) gamma spectrum:

Strontium-85 (0.51 Mev gamma) - Channel 21 - 29

Manganese-54 (0.84 Mev gamma) - Channel 38 - 46

Zinc-65 (1.12 Mev gamma) - Channels 51-59

From gamma spectra for 0.93 μCi strontium-85, 1.07 μCi, manganese-54 and 3.70 μCi zinc-65:

Sm = 1,337 cpm	Zz = 6,133 cpm	Ss = 5,513 cpm
Mm = 5,194 cpm	Sz = 1,974 cpm	Zs = 0 cpm
Zm = 24 cpm	Mz = 1,238 cpm	Ms = 86 cpm

The following ratios are determined:

Fms = 0.257	Fsm = 0.016	Fzm = 0.203
Fmz = 0.005	Fsz = 0.001	Fzs = 0.322

The following equations can be written to express the net observed count:

Net count observed in the strontium-85 area of the gamma spectrum,

$Ns = Ss - Fms\ Mn - Fzs\ Zz$

Net manganese-54 count, $Nm = Fsm\ Ss - Mm - Fzm\ Zz$

Net zinc-65 count, $Nz = Fsz\ Ss - Fmz\ Mm - Zz$

The net count due to the strontium isotope in the region of the strontium peak can be expressed as the determinant:

$$Ss = \frac{\begin{matrix} Ns & Fms & Fzs \\ Nm & 1 & Fzm \\ Nz & Fmz & 1 \end{matrix}}{\begin{matrix} 1 & Fms & Fzs \\ Fsm & 1 & Fzm \\ Fsz & Fmz & 1 \end{matrix}}$$

Likewise the net counts for 54 Mn and 65 Zn can be expressed as determinants:

$$Mm = \frac{\begin{matrix} 1 & Ns & Fzs \\ Fsm & Nm & Fzm \\ Fsz & Nz & 1 \end{matrix}}{\begin{matrix} 1 & Fms & Fzm \\ Fsm & 1 & Fzm \\ Fsz & Fmz & 1 \end{matrix}} \qquad Zz = \frac{\begin{matrix} 1 & Fms & Ns \\ Fsm & 1 & Nm \\ Fsz & Fmz & Nz \end{matrix}}{\begin{matrix} 1 & Fms & Fzs \\ Fsm & 1 & Fzm \\ Fsz & Fmz & 1 \end{matrix}}$$

Expansion of these determinants and substitution of the interference factors yield the following set of simultaneous equations for the net count rates due to 85_{Sr}, 54_{Mn} and 65_{Zn}:

(1) Ss = 1.005 Ns - 0.257 Nm - 0.273 Nz

(2) Mn = 1.005 Nm - 0.06 Ns - 0.199 Nz

(3) Zz = 1.002 Nz - 0.001 Ns - 0.005 Nz

The interference corrected activities stated in the experimental results of this investigation were all obtained through solution of the above set of equations.

APPENDIX C

CALCULATION OF LABORATORY DOSE RATES TO PONTOPOREIA

Internal Gamma-ray Dose-Rate:

Maximum concentrations realized in laboratory animals used in accumulation experiments (page 24) were as follows:

Strontium-85, 4.34×10^{-3} μCi/g;

Manganese-54, 8.60×10^{-3} μCi/g; and

Zinc-65, 4.4×10^{-3} μCi/g (weighted for γ-abundance).

C, total concentration of γ-emitting radionuclides = 17.3×10^{-3} μCi/g

q, total body burden of gamma activity = $17.3 \times 10^{-6} \frac{\text{mCi}}{\text{g}})$ (0.005 g)

$= 8.65 \times 10^{-8}$ mCi

E_γ, average gamma-ray energy $= \frac{(0.51 + 0.84 + 1.14)}{3}$ Mev

= 0.84 Mev

*Gamma-ray dose-rate constant, Γ

$= 170 \frac{\mu_a}{P} E_r$, cm^2 - rad/mCi - hour

where $\frac{\mu_a}{P}$, true mass absorption coefficient of tissue, assumed to be equivalent to water.

$= 0.03\ \text{cm}^2/\text{g}$

$\Gamma = 170\ (0.03)\ (0.84) = 4.30\ cm^2 - rad/mCi - hour$

r, mean radius of the organism = 0.05 cm

*R_γ, absorbed gamma-ray dose-rate to P. affinis in Experiment-4

$= q/r^2$, rads/hour

$$R_\gamma = \frac{\left(4.30\ \frac{cm^2 - rad}{mCi - hour}\right)\left(\frac{8.65 \times 10^{-8}}{mCi}\right)}{(.0025\ cm^2)}$$

$= 1.5 \times 10^{-4}$ rad/hour

Internal Beta Dose-Rate:

Average zinc-65 beta energy, $E_\beta = 0.108$ Mev

C, absorbed beta activity = (Max. amphipod activity X beta abundance) =

$(10^{-2}\ \mu Ci/g \times 0.56) = 5.6 \times 10^{-3}\ \frac{\mu Ci}{g}$

*R_β, absorbed beta dose-rate to P. affinis in Experiment-4 =

$R_\beta = 2.13\ E_\beta C$ rad/hour

$= 2.13\ (0.108)\ (5.6 \times 10^{-3})\ \frac{rad}{hour}$

$= 1.30 \times 10^{-3}$ rad/hour

---

*Dose-rate formula from: Hine, G. J. and Brownell, G. L., Radiation Dosimetry, Academic Press, N. Y. (1956) Chapter 17, 801-870.